Efficacy of vaccination of cattle with the *Leptospira interrogans* serovar *hardjo* type hardjoprajitno component of a pentavalent *Leptospira* bacterin against experimental challenge with *Leptospira borgpetersenii* serovar *hardjo* type hardjo-bovis

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Objective—To evaluate the efficacy of vaccination with the *Leptospira interrogans* serovar *hardjo* type hardjoprajitno component of a pentavalent *Leptospira* bacterin against a virulent experimental challenge with *Leptospira borgpetersenii* serovar *hardjo* type hardjo-bovis strain 203 in cattle.

Animals—Fifty-five 6-month-old Holstein heifers.

Procedures—Heifers that were negative for persistent infection with bovine viral diarrhea virus determined via immunohistochemical testing and negative for *Leptospira interrogans* serovar *pomona*, *Leptospira interrogans* serovar *hardjo*, *Leptospira interrogans* serovar *grippotyphosa*, *Leptospira interrogans* serovar *bratislava*, *Leptospira interrogans* serovar *canicola*, and *Leptospira interrogans* serovar *icterohaemorrhagiae* determined via microscopic agglutination assay were enrolled in the study. Two heifers were separated and used for the challenge passage. The remaining heifers were vaccinated twice with a commercial pentavalent bacterin or a sham vaccine 21 days apart and subsequently challenged with *L borgpetersenii* serovar *hardjo* type hardjo-bovis strain 203. Urinary shedding, antibody titers, and clinical signs of leptospirosis infection were recorded for 8 weeks after challenge.

Results—Heifers that received the pentavalent bacterin did not shed the organism in urine after challenge and did not have renal colonization at necropsy. Heifers that were sham vaccinated shed the organism in urine and had renal colonization.

Conclusions and Clinical Relevance—Results provided evidence that a pentavalent *Leptospira* vaccine containing *L interrogans* serovar *hardjo* type hardjoprajitno can provide protection against challenge with *L borgpetersenii* serovar *hardjo* type hardjo-bovis strain 203. It is important to demonstrate cross-protection that is vaccine specific against disease-causing strains of organisms that are prevalent under field conditions. (*Am J Vet Res* 2012;73:735–740)

Leptospirosis is the most widespread zoonotic disease in the world, affects most mammals, and is an important cause of reproductive failure in cattle throughout the world.[1,2] Leptospires are gram-negative, thin, helical bacteria classified into at least 12 pathogenic and 4 saprophytic species, with > 250 pathogenic serovars. The bacteria may survive in the environment for up to 6 months, especially in basic soils (pH, 7.2 to 8.0) and in a warm, moist environment. The bacteria rarely survive in dry or extremely cold conditions.[1,2]

Pathogenic species persist as chronic infections of the renal tubules of the maintenance host, sometimes causing little or no disease. Transmission occurs through direct contact with infected urine or by contact

Abbreviations

CI	Confidence interval
LHB	*Leptospira borgpetersenii* serovar *hardjo* type hardjo-bovis
LHP	*Leptospira interogans* serovar *hardjo* type hardjoprajitno
MAT	Microscopic agglutination test

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through feed or bedding contaminated with infected urine. In cattle, common clinical signs of the disease include reproductive failure, weak calves, abortion, stillbirths, mummification, and agalactia. Shedding of the bacteria in urine may occur for extended periods. Renal lesions may be the direct result of leptospiremia from infection with *Leptospira interrogans* serovar *hardjo*, *Leptospira interrogans* serovar *pomona*, and *Leptospira interrogans* serovar *grippotyphosa*.[1–3]

Cattle are the primary maintenance host reservoir for LHP and LHB. *Leptospira interogans* serovar *hardjo* type hardjoprajitno is found primarily in Europe, whereas the most common cause of leptospirosis in cattle throughout much of the world is LHB. *Leptospira borgpetersenii* serovar *hardjo* type hardjo-bovis is the only *L borgpetersenii* serovar *hardjo* type isolated from cattle in the United States.[4]

Vaccination with whole-cell inactivated leptospiral vaccine containing *L interrogans* serovar *hardjo*, *Leptospira interrogans* serovar *canicola*, *L interrogans* serovar *pomona*, *L interrogans* serovar *grippotyphosa*, and *Leptospira interrogans* serovar *icterohaemorrhagiae* is the primary means of controlling leptospirosis in cattle. The purpose of the study reported here was to evaluate efficacy of the LHP component of a commercial pentavalent-adjuvanted combination vaccine against a virulent challenge with LHB. Efficacy was determined on the basis of reduction of renal infection, colonization, and urinary shedding of the challenge strain.

Materials and Methods

Animals—All procedures involving cattle were approved by the Animal Care and Use Committee of Rural Technologies Inc. Ten days prior to vaccination, fifty-five 6-month-old Holstein heifers arrived at a drylot housing facility. All animals were healthy, had no history of vaccination against *Leptospira* spp, and were serologically negative to 6 serovars of *Leptospira* (*L interrogans* serovar *pomona*, *L interrogans* serovar *hardjo*, *L interrogans* serovar *grippotyphosa*, *L interrogans* serovar *bratislava*, *L interrogans* serovar *canicola*, and *L interrogans* serovar *icterohaemorrhagiae*) as determined with a serologic *Leptospira* MAT. In addition, all 55 animals were negative for bovine viral diarrhea virus via ear-notch immunohistochemical testing. Fifty-five animals were initially procured to ensure that a minimum of 50 animals would be available for the vaccination and challenge portions of the study as well as 2 animals for the passage of the challenge material.

Animal housing and feeding—On arrival at the housing facility, animals were group housed in a 3,135 m^2 drylot with a 45.7-m concrete fence-line feed bunk. Animals were moved as a group into a hoop barn (approx 379 m^2) prior to challenge. The hoop barn facility provided 30.5 m of feed bunk-line space with head locks as well as 2 automatic waterers. All animals were fed an age-appropriate grain ration with grass hay and provided water ad libitum. Additionally, feed was used to entice the heifers into the head locks on the days the animals were subjected to procedures.

Masking—The investigator or designees performing postchallenge clinical evaluations were unaware of individual assignments to treatment groups. Laboratory personnel testing serum, urine, and tissue samples were also unaware of the group assignments.

Animal enrollment, randomization, and animal observations—The assignment of animals to groups prior to the initial vaccination followed a completely randomized distribution. The treatments included a modified-live virus and adjuvanted bacterin combination vaccine[a] (group 1), a killed adjuvanted virus bacterin combination vaccine[b] (group 2), and a placebo control group vaccinated with modified-live virus vaccine without the *Leptospira* bacterin[c] (control group).

The heifers were assigned to groups with a random numbers generator.[d] On day 0, 53 animals were randomly assigned to 1 of 3 groups. Two heifers were randomly selected as challenge passage animals. At the end of the study, enrolled heifers were randomly assigned to 1 of 2 necropsy dates with the random numbers generator as described.

Treatment groups—Vaccinates were administered a modified-live viral-bacterin combination product consisting of bovine rhinotracheitis virus, bovine viral diarrhea virus, parainfluenza 3, and bovine respiratory syncytial virus vaccine (modified-live virus) containing *Campylobacter fetus*, *L interrogans* serovar *canicola*, *L interrogans* serovar *grippotyphosa*, *L interrogans* serovar *hardjo*, *L interrogans* serovar *icterohaemorrhagiae*, and *L interrogans* serovar *pomona* bacterin[a] with an adjuvanted *Leptospira* fraction (group 1) or were administered a killed adjuvanted virus combination product consisting of bovine rhinotracheitis virus, bovine viral diarrhea virus, parainfluenza 3, and bovine respiratory syncytial virus vaccine (killed virus) containing *C fetus*, *Haemophilus somnus*, *L interrogans* serovar *canicola*, *L interrogans* serovar *grippotyphosa*, *L interrogans* serovar *hardjo*, *L interrogans* serovar *icterohaemorrhagiae*, and *L interrogans* serovar *pomona* bacterin[b] (group 2). The placebo-vaccinated control group was administered a modified-live virus vaccine consisting of bovine rhinotracheitis virus, bovine viral diarrhea virus, parainfluenza 3, and bovine respiratory syncytial virus.[c]

The products used for vaccination of group 1 and the control group were fully released commercially available products. The product used for vaccination of group 2 was an experimental serial of a product prepared specifically for use in the present study. All vaccinations were administered via the SC route. The test vaccines and placebo were administered as 2 doses, each at 2 or 5 mL, with a 21-day interval between the vaccinations. The test vaccines were formulated to the minimum immunizing dose of organisms for the *L interrogans* serovar *hardjo* component. All remaining components were prepared at or greater than the manufacturer's recommended dose concentration.

Animal health examination—All animals were examined by a veterinarian and considered healthy on day 0, prior to sample collection.

Vaccination—Animals in groups 1 and 2 and the control group were vaccinated on postvaccination days 0 and 21. Animals were observed daily for 7 days following each vaccination for postvaccination reactions.

No postvaccination adverse effects were seen in any of the animals.

Blood sample collection—Blood samples were collected from all animals on postvaccination days 0, 21, 35, 43, 105, 112, 119, 126, 133, 140, 147, 154, and 160 via jugular venipuncture. Tubes were labeled with the date and the animal's identification number and returned to the laboratory for processing. The samples were allowed to clot at room temperature (approx 23°C), centrifuged at 800 × *g* for 15 minutes, and aliquoted.

MAT—Serum samples were processed as described, and an aliquot was submitted to a laboratory[e] for *Leptospira* MAT testing to determine the antibody response to *L interrogans* serovar *canicola*, *L interrogans* serovar *grippotyphosa*, *L interrogans* serovar *hardjo*, *L interrogans* serovar *icterohaemorrhagiae*, *L interrogans* serovar *bratislava*, and *L interrogans* serovar *pomona*. The test was performed by adding known live cultures of *Leptospira* to equal amounts of diluted serum and observing the mixture microscopically for agglutination at the end of the reaction time. The endpoint titer was the highest serum dilution with an agglutination of at least 50% of the leptospires in the test well. On the basis of the MAT procedure, animals were considered to be seropositive when the MAT endpoint titer was > 100. Strain LHP was used in the *L interrogans* serovar *hardjo* MAT.

Urine collection—Two 50-mL conical vials of urine were obtained from each heifer on postvaccination days 0, 105, 112, 119, 126, 133, 140, 147, 154, and 160. Samples were collected during midstream urination. The samples were obtained after administering 500 mg of furosemide[f] IV to each heifer. Following collection, the outside of each vial was disinfected and labeled with the animal's identification number and then all vials were transported to a laboratory[g] for processing for *Leptospira* culture.

Bacteriologic culture of urine—The urine samples were used to detect leptospires via bacteriologic culture of urine. Briefly, 1 mL of urine from each heifer was serially diluted 1:10 in transport medium to the 10^{-2} dilution. Sample volumes of 100 and 300 µL from each dilution were inoculated in Ellis (80/40) culture medium and incubated at 29°C for up to 2 months. Cultures were observed microscopically for signs of *Leptospira* growth. Culture samples with bacterial growth were confirmed to have *Leptospira* organisms by means of dark-field microscopy. Cultures with no growth after 2 months of observation were considered to have negative results.

Bacteriologic culture of kidney samples—Immediately following euthanasia, both kidneys were removed from each heifer. Tissue samples totaling approximately 1 cm^2 (approx 1 g) of tissue from both kidneys were collected from each heifer. If suspect areas of the kidney were noted, characterized by pale areas of depression on the surface of the kidney, tissue samples were obtained from those areas. If no suspect areas were seen, samples were obtained at random from each kidney. Each kidney sample was homogenized, and the kidney homogenates were diluted in growth medium and incubated at 29°C for up to 2 months. Cultures were observed microscopically for signs of *Leptospira* growth. Culture samples with bacterial growth were confirmed to have *Leptospira* organisms by means of dark-field microscopy. Cultures with no growth after 2 months of observation were considered to have negative results.

Production of challenge inoculum—On postvaccination day 25, the 2 untreated heifers used for production of challenge inoculum were moved to a separate building at the same facility for challenge. The animals were maintained on the same ration as the remaining groups and allowed free access to grass hay. Water was provided ad libitum.

The LHB strain 203[h] designated for the challenge has been described.[4–6] The 2-passage heifers were challenged on postvaccination days 25, 26, and 27. Each heifer was challenged intraocularly on each challenge day with approximately 1.4×10^7 organisms from freshly grown LHB culture contained in 1.0-mL inoculum. The animals were restrained in a chute, and each head was secured with a halter. Animals were challenged intraocularly by pulling the lower eyelid down to expose the conjunctival sac. A volume of 0.5 mL of challenge material was dropped into each sac, 1 eye at a time, and each eyelid was held closed for 60 seconds following administration of the challenge material.

Blood and urine samples were obtained from the 2-passage heifers on days 0, 15, 23, 28, 36, 42, and 51 after the first challenge to follow the infection's progress. Urine samples collected from both animals were tested for the presence of the organism. The culture result for one heifer was positive for leptospires in the urine beginning the third week following challenge and continuing until necropsy; the results of bacteriologic culture of urine in the other heifer were positive for leptospires on the sixth and seventh week following challenge. On day 51 after the first challenge, these 2 heifers were euthanized by IV administration of a barbiturate[i] overdose.

The kidneys were removed from the animals and transported to the laboratory[g] for processing and *Leptospira* isolation. Suspect lesions from each kidney of one heifer were removed, weighed, and homogenized before adding to the culture media; no suspect lesions were found on the kidneys from the other heifer; thus, random sections were removed, weighed, and homogenized before adding to the culture media. The kidney homogenates were diluted in growth medium for bacteriologic culture of kidney samples, which were grown under the same conditions as the urine as described.

The kidney samples obtained at the time of necropsy from one heifer had positive culture results for leptospires, but those from the other heifer had negative results. The positive kidney cultures were passaged into fresh growth medium to produce the challenge inoculum. The challenge inoculum was checked weekly to determine organism growth and motility. The challenge inoculum was considered ready when motility was high and sufficient culture was available for 3 challenges of all study animals with a 1.0-mL inoculum containing approximately 10^6 organisms/mL. The identities of the

organisms as *Leptospira* spp were confirmed with darkfield microscopy.

Vaccination and challenge phase—Animals in groups 1 and 2 and the control group were vaccinated on postvaccination days 0 and 21. No adverse effects of vaccination were seen in any of the animals.

Leptospira borgpetersenii serovar *hardjo* type hardjo-bovis strain 203 was grown in the laboratory[g] following the 2-animal challenge passage phase and was used for challenge. On postvaccination days 105, 106, and 107, fresh material was counted on a hemacytometer and aliquoted into 3-mL syringes, each containing 1 mL of challenge material at approximately 10^6 leptospires/mL. Additionally, a postchallenge sample was returned to the laboratory[g] and counted on a hemacytometer. The aliquots were used to challenge the heifers in groups 1 and 2 and the control group. Briefly, the animals were restrained and challenged intraocularly by pulling the lower eyelid down to expose the conjunctival sac. A volume of 0.5 mL of challenge material was dropped into each sac, 1 eye at a time. Each eyelid was held closed for 60 seconds following administration of the challenge material. The animals were observed once daily for general clinical signs from the day of challenge to the day of necropsy.

Blood and urine sample collection—Blood samples were collected via jugular venipuncture from all animals just prior to challenge and weekly from days 7 to 55 after challenge. The samples were processed as described, and an aliquot was submitted to the laboratory[e] for *Leptospira* MAT testing. Two 50-mL conical vials of urine were obtained from each animal once weekly from day 0 to 55 after challenge. Samples were collected as described. The urine samples were used to detect leptospires via bacteriologic culture of urine as described.

Animal disposition and tissue sample collection—On postvaccination days 161 and 162 (56 and 57 days after challenge), animals (n = 53) were transported to the laboratory[e] for necropsy and tissue sample collection. Approximately half of the heifers were randomly assigned to undergo necropsy on day 161, and the second half was assigned to undergo necropsy on day 162. The animals were euthanized by IV administration of a barbiturate overdose. Immediately following euthanasia, both kidneys were removed from each carcass. Suspect lesions were obtained from the kidneys. If no suspect lesions were observed, random samples were collected from the kidneys. The samples were weighed and homogenized prior to addition to the culture media. The cultures were grown under the same conditions as the bacteriologic cultures of urine.

Statistical analysis—The 53 animals were randomly assigned to treatment groups, with 21 heifers in both groups 1 and 2 and 11 animals in the control group. Urine samples were evaluated for organism recovery on postvaccination days 0, 105 (0 days after challenge), 112 (7 days after challenge), 119 (14 days after challenge), 126 (21 days after challenge), 133 (28 days after challenge), 140 (35 days after challenge), 147 (42 days after challenge), 154 (49 days after challenge), and 160 (55 days after challenge). Kidney tissue samples were evaluated for organism recovery following necropsy.

Prevented fractions were used to compare organism recovery for urine shedding and bacteriologic culture of kidney samples between the vaccine and control groups. Organism recovery for urine shedding was defined as the presence of at least 1 positive culture during the study or no positive culture on any study day. Organism recovery for kidney tissue samples was defined as a positive culture or a negative culture.

The estimated prevented fraction (pf) was calculated as the complement of the risk ratio and was defined as follows: $pf = 1 - (y_2/n_2)/(y_1/n_1)$, where y_2 is the number affected in the treated group, n_2 is the total number in the treated group, y_1 is the number affected in the control group, and n_1 is the total number in the control group.

Statistical software[j] was used to compute the exact 95% CI of the risk ratio on the basis of the standardized statistic and inverting two 1-sided tests. The 95% CI for the prevented fraction is the complement of the 95% CI for the risk ratio. The corresponding 2-sided value was reported. The *P* value for the exact test for the prevented fraction (0 [the null hypothesis]) is the same as the *P* value for the exact test for the risk ratio (1). The entire 95% CI is > 0.0% when the prevented fraction is significantly ($P < 0.05$) different from 0.0%.

Values of $P < 0.05$ were considered significant for all hypothesis testing. Zero and near-zero values for the number of animals affected in the groups being compared can result in unstable 95% CIs and *P* values.

Results

MAT results—Blood samples were obtained from all enrolled heifers in groups 1 and 2 and from the control group on postvaccination days 0, 21, 35, and 43 for MAT testing to determine the antibody response to *L interrogans* serovar *canicola*, *L interrogans* serovar *grippotyphosa*, *L interrogans* serovar *hardjo*, *L interrogans* serovar *icterohaemorrhagiae*, and *L interrogans* serovar *pomona*. Transient postvaccination MAT serologic responses were detected to *L interrogans* serovar *canicola*, *L interrogans* serovar *grippotyphosa*, *L interrogans* serovar *icterohaemorrhagiae*, and *L interrogans* serovar *pomona* in 40% to 75% of the vaccinated heifers on days 14 and 21 after the second vaccination in both vaccination groups (data not shown). A transient positive MAT serologic response to the *L interrogans* serovar *hardjo* was observed only on day 14 after second vaccination in < 24% of the vaccinates. All unvaccinated controls remained negative to all serovars until the day of challenge.

Postchallenge blood samples were obtained from the control group and from all enrolled heifers in groups 1 and 2 on days 0, 7, 14, 21, 28, 35, 42, 49, and 55 after challenge for MAT testing to determine their antibody response to *L interrogans* serovar *canicola*, *L interrogans* serovar *grippotyphosa*, *L interrogans* serovar *hardjo*, *L interrogans* serovar *icterohaemorrhagiae*, and *L interrogans* serovar *pomona*. Postchallenge MAT antibody responses remained negative for *L interrogans* serovar *canicola*, *L interrogans* serovar *grippotyphosa*, *L interrogans* serovar

Table 1—Prevented fraction and exact 95% CIs for bacterial recovery in a study of efficacy of vaccination against LHB in cattle.

Sample	Treatment group	Prevented fraction	Exact *P* value	Lower 95% CI	Upper 95% CI
Urine	1 vs 3	1.000	< 0.001	0.839	1.000
	2 vs 3	1.000	< 0.001	0.839	1.000
Kidney	1 vs 3	1.000	< 0.001	0.835	1.000
	2 vs 3	1.000	< 0.001	0.835	1.000

icterohaemorrhagiae, and *L interrogans* serovar *pomona* in all animals until the end of the study. Postchallenge MAT results for *L interrogans* serovar *hardjo* remained negative in all vaccinated heifers from groups 1 and 2 until the end of the study. However, in control heifers, postchallenge MAT antibody response was evident in 9 of 11 animals by day 14 after challenge and in 11 of 11 animals by day 21 after challenge. Peak MAT titers in the unvaccinated controls ranged from 800 (7/11 heifers) to 1,600 (4/11 heifers; data not shown).

Urine shedding results—All prechallenge and postchallenge bacteriologic cultures of urine from all urine samples collected from the vaccinated heifers (groups 1 and 2) were negative for growth of leptospires. This was in contrast to control heifers, of which 100% had positive results of bacteriologic culture of urine with a minimum of 2 postchallenge samples. The range was 2 to 6 positive cultures/heifer. Urine shedding was first detected in control heifers on day 21 after challenge, with the highest number of animals shedding on days 35 and 49 after challenge.

The calculated prevented fraction for urine shedding for each vaccine group, compared with the control group, was 1.000 (100%; exact 95% CI, 0.839 to 1.000 [range 83.9% to 100%]; **Table 1**). No animals in either vaccine group had any positive results from bacteriologic culture of urine, and all animals in the control group had at least 2 positive results.

Results from bacteriologic culture of kidney samples—All vaccinated heifers (groups 1 and 2) were negative for leptospire isolation from kidney tissues, and 10 of 11 heifers in the control group had positive leptospire results from bacteriologic culture of kidney tissues.

The calculated prevented fraction results from bacteriologic culture of kidney tissue samples for each vaccine group, compared with results for the control group, was 1.000 (100%; exact 95% CI, 0.835 to 1.000 [range, 83.5% to 100%]; Table 1). No heifers in either vaccine group had any positive culture results, and 10 of 11 heifers in the control group had positive results from bacteriologic culture of kidney samples.

Discussion

The objective of the present study was to determine the ability of the LHP component of 2 combination vaccines to protect against challenge with LHB. Postchallenge culture results indicated that none of the vaccinated heifers in groups 1 and 2 had infection as a result of the challenge. This was in sharp contrast to the control animals, of which 100% became infected.

The MAT serologic results revealed transient seroconversion after the second vaccination in a portion of the vaccinated animals for all serovars. All animals in each group were negative for all serovars at the time of challenge as determined with the MAT. By 3 weeks after challenge, all control animals were MAT positive for *L interrogans* serovar *hardjo* as a response to challenge and resulting infection with the challenge organism. Challenge of the animals with the LHB did not elicit an anamnestic MAT serologic response to *L interrogans* serovar *hardjo* in the vaccinated animals, whereas all 11 controls had positive MAT results after challenge. The challenge organism was recovered via bacteriologic culture of urine samples collected at weekly intervals until day 55 after challenge. Control animals each had a minimum of 2 urine samples that had tested positive via bacteriologic culture of urine, indicating shedding for at least 7 days. The duration of shedding in the control animals ranged from 7 to 35 days. Of 11 control heifers, 1 shed for 2 sampling days, 4 for 4 days, 4 for 5 days, and 2 for 6 days. No leptospires were recovered from any urine sample in any of the vaccinated animals. On the basis of the culture results, vaccination with the *L interrogans* serovar *hardjo* bacterin completely prevented urinary shedding of the challenge organism.

Kidney samples obtained at the time of necropsy were also examined via bacteriologic culture to determine challenge organism recovery. Kidney tissues from 10 of 11 of the controls were positive for recovery of the challenge organism. There was no recovery of the challenge organism from any kidney tissues for any of the vaccinated heifers (groups 1 and 2). On the basis of culture results, vaccination with the *L interrogans* serovar *hardjo* bacterin completely prevented kidney infection and colonization with the challenge organism.

Challenge of the heifers vaccinated with either the modified-live combination product or the killed viral combination product with the LHB did not elicit an anamnestic MAT serologic response to *L interrogans* serovar *hardjo*, whereas all 11 of the placebo controls were MAT positive after challenge. These results supported the notion that the MAT serologic response of control heifers increased as a result of the active infection. This was in sharp contrast to the absence of an MAT serologic response after challenge in the vaccinated animals. The lack of MAT serologic response in the vaccinated animals could simply indicate that replication of the challenge organism did not occur in these vaccinated animals, at least to an extent sufficient to elicit an anamnestic serologic response. Authors of other studies[7,8] have also reported the lack of a postchallenge anamnestic serologic response from *Leptospira*-vaccinated animals and have discussed the possibility of a blocking effect of IgG on the epitopes of the immunogen or a negative feedback effect on B-lymphocyte receptors that prevents a secondary antibody response.[7] Historically, protective immunity against *L interrogans* serovar *hardjo* was thought, primarily on the basis of passive protection studies[9,10] that used antibodies against leptospiral lipopolysaccharides, to be antibody mediated. However, studies[5,6] involving challenge of animals with high titers of antilipopolysaccharide antibody at the time of challenge found that the animals

were not protected against challenge with *L interrogans* serovar *hardjo*. Results of previous studies[11,12] that used vaccines to provide protection for cattle against challenge with LHB strain 203 reveal that the induction of a potent postvaccination Th1–type immune response involving CD4 and λδ T lymphocytes may be associated with vaccine-induced protection against challenge. Perhaps the strong protective memory aspect provided by the Th1 cell–mediated immune response in the vaccinated animals is able to prevent replication of the challenge organism, the result of which may be insufficient antigen available for recognition by the immune system of vaccinated and challenged animals, which would result in induction of an anamnestic antibody-based response.

Results of the present study were similar to those of a vaccine study,[8] which found a monovalent leptospiral vaccine provided protection against challenge with LHB strain 203. The leptospiral vaccine in that study[8] was reported to be prepared from LHB strain 93U, whereas the strain used in preparation of the vaccine for the study reported here was LHP. These are 2 serologically identical but genetically distinct types of *L interrogans* serovar *hardjo*.[13–16] In contrast to the present study, previous studies[4–6,8] found that vaccines containing LHP do not protect well against urine shedding or renal infection when vaccinated animals are challenged with LHB. Subtle differences in the *L interrogans* serovar *hardjo* used to prepare the vaccine may, in part, account for the level of protection provided by this vaccine. This may include differences in the original isolate as well as differences induced on the basis of methods of growth and passage. Growth and inactivation conditions of the strain can also impact the efficacy provided by the vaccine. Serovar differences combined with organism processing for the vaccine will affect the ability of the vaccine to induce a protective immune response.

Vaccination with both the modified-live viral-LHP bacterin combination and killed viral LHP bacterin combination resulted in prevention of urinary shedding as well as prevention of renal infection and colonization after challenge with LHB. The results of the present study were supportive of the conclusion that the *L interrogans* serovar *hardjo* component of the test vaccines is efficacious against challenge-induced renal infection and colonization and associated urinary shedding of LHB.

a. Express FP 5 VL5, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
b. Unlicensed experimental combination product, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
c. Express FP 5, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
d. Analysis ToolPak, Microsoft Excel, Microsoft Corp, Redmond, Wash.
e. South Dakota Animal Disease Research and Diagnostic Laboratory, Veterinary and Biomedical Sciences Department. South Dakota State UniversityBrookings, SD.
f. Furosemide, Vedco Inc, St Joseph, Mo.
g. Rural Technologies Inc Laboratory, Brookings, SD.
h. National Animal Disease Center, Ames, Iowa.
i. Euthanasia 6 Grain, Vedco Inc, St Joseph, Mo.
j. StatXact, version 8.0, Cytel Software Corp, Cambridge, Mass.

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