Effect of a monovalent vaccine against *Leptospira borgpetersenii* serovar Hardjo strain hardjobovis on fertility in Holstein dairy cattle

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**Objective**—To determine whether vaccination with a monovalent vaccine against *Leptospira borgpetersenii* serovar Hardjo strain hardjobovis would improve reproductive efficiency in Holstein cattle in a commercial dairy setting.

**Design**—Randomized controlled trial.

**Animals**—1,894 Holstein cows and heifers from a Central California dairy.

**Procedures**—Cattle were assigned to undergo SC administration of a monovalent vaccine against *Leptospira borgpetersenii* serovar Hardjo strain hardjobovis (n = 986) or a placebo (lactated Ringer’s solution; 908). At the end of their lactation period, cows received 2 doses of the vaccine or placebo, 28 to 35 days apart, with the initial dose administered in conjunction with oxytetracycline. Heifers received the same treatments, with the second dose administered at least 2 weeks before their entrance into the heifer breeding pen. Urine and blood samples were collected from randomly selected cattle immediately before and 1 year after the trial began and submitted for fluorescent antibody and microscopic agglutination testing to identify any infecting *Leptospira* serovar.

**Results**—The initial herd prevalence of active infection with strain hardjobovis was 13% (6/46 tested cattle), followed by 15% (6/40) 1 year after the trial began. The odds of heifers conceiving over the period at risk for conception, regardless of vaccination, was approximately 2.8 times as high as for primiparous and pluriparous cows. Survival analysis of days from parturition to conception revealed that the vaccine protocol had no effect on the probability of conception between the vaccinated and control groups. The vaccine protocol had no impact on pregnancy loss.

**Conclusions and Clinical Relevance**—The evaluated vaccination protocol against *Leptospira* strain hardjobovis was not effective in improving reproductive efficiency in commercial Holstein dairy cows or in decreasing urine shedding of leptospires. (J Am Vet Med Assoc 2013;242:1564–1572)

Leptospirosis, a zoonotic disease caused by a gram-negative bacterial spirochete, adversely affects reproductive efficiency in dairy cattle by resulting in lower than typical conception rates, failure to conceive, and embryonic and fetal death. More than 200 serovars of *Leptospira* have been identified worldwide, many of which are pathogenic to cattle. Most infections in cattle are attributed to serovar Hardjo, of which 2 strains are known to exist: *Leptospira interrogans* serovar Hardjo strain hardjoprajitno and *Leptospira borgpetersenii* serovar Hardjo strain hardjobovis. The 2 strains of serovar Hardjo are serologically and clinically similar yet genetically and antigenically distinct. Strain hardjobovis, known as the US reference strain, was believed to be the primary leptospiral pathogen of North American cattle prior to 1990; however, advances in diagnostic techniques led to the discernment of genetic differences within serovar Hardjo and its subsequent classification into 2 species and strains. Strain hardjobovis has been isolated from cattle populations worldwide, including those in the...
United States, whereas strain hardjoprajitno appears to be restricted primarily to the United Kingdom.\textsuperscript{1,10,11} Prevalence estimates of strains hardjoprajitno and hardjobovis in the United Kingdom are not readily available; however, strain hardjoprajitno is reportedly endemic in UK cattle.\textsuperscript{2,12} Strain hardjobovis is believed to be the most common \textit{Leptospira} strain in cattle in Australia, New Zealand, the United Kingdom, and North America.\textsuperscript{13,14} with 42% of beef herds\textsuperscript{15} and 57% of dairy herds\textsuperscript{16} in the United States having positive test results in 2007 and 2003, respectively.

Cattle serve as maintenance hosts for strains hardjoprajitno and hardjobovis; therefore, the rate of transmission and incidence of infection with these particular strains is high.\textsuperscript{10} \textit{Leptospira} penetrate mucous membranes\textsuperscript{1} via fluids such as contaminated drinking water and excretions from infected animals such as urine, milk, and placental fluids.\textsuperscript{10,17} The organisms circulate in the blood and can invade all major organs. Colonization is most common in the kidneys and reproductive tract, allowing shedding of \textit{leptospiras} into urine, which increases the risk of transmission to others. \textit{Spirochete} replication in the reproductive tract increases the potential for venereal and transplacental transmission,\textsuperscript{17} whereas persistent infection of the reproductive tract can lead to early embryonic death, abortion, stillbirth, and birth of weak calves.\textsuperscript{3,17,18}

Persistent infection of the reproductive tract in female cattle is the most economically important manifestation of serovar Hardjo.\textsuperscript{1,17} Although the mechanisms are not fully understood, researchers presume that after bacteremia and bacteriuria, \textit{spirochetes} can persist in the oviduct and uterus, interfering with embryo implantation and other early pregnancy events.\textsuperscript{1} This causes infertility by failure to maintain the conceptus and is associated with an increase in number of services per conception and a prolonged calving interval.\textsuperscript{1} Estimated costs of $2.55/d to $4.68/d are incurred when a cow fails to conceive during the optimal calving interval.\textsuperscript{19,20} In a dairy herd, the cumulative results of failure to conceive or maintain the conceptus are a decrease in the dairy’s profitability.

Annual vaccination and antimicrobial administration have been used for prevention and treatment of leptospirosis in cattle.\textsuperscript{37} Because the 2 strains of serovar Hardjo are serologically similar and results of serologic testing have been classically used to determine the organisms used for vaccine development, the US reference strain hardjoprajitno has been used in available pentavalent vaccines for North American cattle. The commercially available pentavalent vaccines, which target strain hardjoprajitno, have not protected against strain hardjobovis because the vaccines do not contain hardjobovis antigens and cross-protection between strains does not develop.\textsuperscript{3,21}

Research into the efficacy of a commercially available monovalent strain hardjobovis vaccine has been conducted; however, findings were inconclusive.\textsuperscript{3,22,23} In a small study\textsuperscript{22} of vaccinated beef heifers experimentally challenged with strain hardjobovis, renal and uterine tissue colonization with \textit{leptospiras} was prevented and urinary shedding of \textit{leptospiras} was eliminated. Although it is feasible that the heifers would also have a lower likelihood of reproductive tract colonization with the organism than would cows, reproductive performance was not assessed. A larger study\textsuperscript{23} of naturally infected dairy cattle that received the same monovalent hardjobovis vaccine resulted in an increase in conception rates at first service; however, the specific strain of \textit{Leptospira} was not identified and the reproductive efficiency variables evaluated were of limited scope, compared with those evaluated in similar studies. A third study\textsuperscript{1} of beef cattle that received the monovalent vaccine coupled with long-acting oxytetracycline revealed no significant improvement in reproductive performance in naturally infected herds.

The effects of antimicrobial use for treatment of infection with \textit{Leptospira} strain hardjobovis were also evaluated in experimentally infected cattle.\textsuperscript{17} That study showed that several antimicrobials were suitable for eliminating renal colonization and urinary shedding of \textit{leptospiras}, including injection once with oxytetracycline, although cattle were only monitored for 4 to 6 weeks and resistance to reinfection was not evaluated. The mixed results and various trial protocols used to evaluate the efficacy of a monovalent strain hardjobovis vaccine in US dairy cattle clearly indicate the need for additional research. The objective of the study reported here was to determine whether a monovalent vaccine against \textit{L. borgpetersenii} serovar Hardjo strain hardjobovis would improve reproductive efficiency in Holstein cattle in a commercial dairy setting.

**Materials and Methods**

**Animals**—Cattle used in the study consisted of a herd (n = 3,600) at a commercial dairy farm in Lodi, Calif, that was considered representative of a typical production dairy environment in the western United States. The herd included 1,900 milking and nonlactating cows. The study protocol and all procedures and animal handling methods were approved by the University of California-Davis Institutional Animal Care and Use Committee. In addition, consent for use of all cattle enrolled in the study was obtained from the dairy herd owner.

**Management**—Cows were housed in free-stall barns with access to open dry lots, fed a total mixed ration, and milked 2 times/d. For the study, the farm’s herd health protocol was not modified. Vaccines in the herd health plan, including those against common abortifacient agents, continued to be administered as was routine. Specifically, at the 30th to 50th day of lactation (30 to 50 DIM), cows received modified live virus vaccines\textsuperscript{5} SC for protection from infectious bovine rhinotracheitis, bovine viral diarrhea, bovine parainfluenza-3, bovine respiratory syncytial virus, and leptospirosis. The \textit{Leptospira} vaccine included 5 serovars (\textit{L. interrogans} serovars Canicola, Grippotyphosa, Hardjo strain hardjoprajitno, \textit{Icterohaemorrhagica}, and \textit{Pomona}). Killed virus vaccines\textsuperscript{5} against the agents of these same diseases were administered SC at confirmation of pregnancy and the cessation of lactation. In addition, \textit{Escherichia coli} bacteria (\textit{Clostridium chauvoei, Clostridium septicum, Clostridium haemolyticum, Clostridium novyi, Clostridium sordellii, and Clostridium perfringens})
types C and D bacterin, and Fusobacterium necrophorum bacterin were administered SC at cessation of lactation (ie, 8 weeks before parturition) and at 3 weeks before parturition and E coli bacterin was readministered at parturition.

Specific vaccines were also routinely administered as part of the heifers’ herd health plan. At birth, heifer calves were given intranasal vaccine for protection against infectious bovine rhinotracheitis and influenza type 3 viruses. At approximately 4 and 5 months of age, heifers were administered SC the same modified-live vaccine as the cows received as well as C chauvoei, C septicum, C haemolyticum, C novyi, C sordellii, and C perfringens types C and D bacterin and a trivalent vaccine consisting of a Moraxella bovis bacterin. In addition, at 4 months of age, a strain RB51 vaccine against Brucella abortus was administered SC.

Determination of leptospirosis herd prevalence—The herd prevalence of leptospirosis due to strain hardjobovis within the herd was determined at 2 points: before and after the vaccine trial. The sample size was determined with the assumption that 20% or none of the herd would be infected, and 42 cow cattle would be needed to determine herd prevalence with a 99.9% CI in a population of 2,000 cattle. Herd positivity was defined such that if 1 animal was identified as infected, the herd would be considered endemic infected. Initially, a subgroup (n = 46) of cows (23) and heifers (23) was selected as a convenience sample from 3 age groups of cattle within the dairy facility: nulliparous heifers, primiparous cows, and pluriparous cows were used. After the trial concluded (at least 82 days after vaccination and antimicrobial treatment or placebo administration), a subgroup (n = 40) of cattle was randomly selected from the vaccinated group (24) and the control group (16) for prevalence testing. This second sample consisted of heifers (n = 12), first and second lactation cows (18), and third or later lactation cows (10).

Two types of samples were obtained for disease detection at both time points. Blood samples were collected from the coccygeal vein. To obtain urine samples, furosemide was injected into the coccygeal vein (1.0 mg/kg), and urine was collected midstream by free catch. Urine and blood samples were submitted to a commercial diagnostic laboratory for Leptospira testing. The presence of leptospires in the urine samples was determined by fluorescein antibody staining as implemented and described elsewhere. When a positive test result was obtained, the presence of anti-leptospiral antibodies in serum harvested from the blood samples was analyzed by MAT to identify the Leptospira serovars.

Titers of antibodies against L interrogans serovars Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagica, and Pomona were measured via MAT by use of serum harvested from the blood samples. When cattle had leptospires identified in their urine and antibody titers for all tested serovars except Hardjo were ≤ 1:100 and for Hardjo were ≥ 100, the cattle were considered to be infected with L borgpetersenii serovar Hardjo strain hardjobovis. In other words, infected cattle as defined for the study were those that were leptospiuric, without high antibody titers for serovars of L interrogans but with an antibody response to Hardjo greater than or equal to that of the other L interrogans species and ≥ 100.

Experimental design—Holstein cows and heifers were selected from the herd for participation in the vaccine trial from June 2009 through June 2011. To be considered for inclusion, all cattle were required to have completed both treatments (vaccinated and antibiotic or placebo, at least 3 weeks apart) at least 2 weeks before breeding. Uniparous or multiparous cows were required to have stayed in the herd and have been present at least 1 day after the voluntary waiting period for breeding (48 days after calving or more), and nulliparous heifers were required to have been bred once. Unbred nulliparous heifers were excluded because they might have had anatomic or physiologic anomalies preventing conception. These criteria yielded 1,894 eligible cattle for random assignment via coin toss to either the vaccinated or control group. Cows were enrolled at cessation of lactation and nulliparous heifers at the time of first breeding.

Cattle in the vaccinated group were inoculated SC with 2 mL of a monovalent Leptospira vaccine containing chemically inactivated whole cultures of L borgpetersenii serovar Hardjo strain hardjobovis. In addition, an SC injection of oxytetracycline (20 mg/kg) was administered as recommended for control of hardjobovis. Vaccinated cattle were revaccinated between 28 and 35 days after the first vaccination, as per label instructions. The second vaccine was administered at least 2 weeks before breeding, as recommended by the manufacturer. Cattle in the control group were given 2 doses (2 mL each) of lactated Ringer’s solution (placebo) SC at the same timing as in the vaccinated group.

Both groups were housed together in pens on the basis of their lactation and stage of lactation with free-stall barns and received additional standard herd health care. All dairy personnel, including the breeder and veterinarian, were unaware of study group assignment; no cattle markings or records were used on the farm to identify study vaccination status.

Lactating cattle were eligible for artificial insemination 44 days after parturition (ie, 44 DIM), and heifers were eligible after introduction to the heifer breeding pen. The observation period for all cows (primiparous and pluriparous) began once they received their second Leptospira vaccination or placebo injection and were eligible for insemination. The observation period for heifers (nulliparous females) began at their initial breeding date. This method was chosen to give the nulliparous heifers sufficient time to begin first estrus (puberty) and reach sufficient body frame size. The observation period continued for a maximum of 1 year after treatment. Cows were visually monitored for signs of estrus by tail chalk removal in accordance with industry standard. Ovulation in cows in which estrus was not detected by 60 days after parturition or cows deemed nonpregnant at the time of pregnancy diagnosis was synchronized with a standard protocol. All cows and heifers were artificially inseminated when estrus was observed. Pregnancy diagnosis was performed as usual by manual and ultrasonic reproductive examination per rectum by the herd veterinarian at 32 to 40 days after insemination. Females declared
to be nonpregnant at a reproductive examination subsequent to a positive pregnancy test result were considered to have aborted. All reproductive records were maintained on dairy computer software and used as the data source.

Statistical analysis—Dairy reproductive records for enrolled animals were used for statistical analysis with the aid of statistical software. Survival analysis, a regression technique for which the time to an event is the measured outcome, was used to measure the effect of the vaccine protocol on interval to conception. When a cow or heifer failed to conceive, that animal was censored at the time of death or culling or at study termination. Cox proportional hazards regression was used to analyze the effect of the vaccine protocol on interval to conception while adjusting for potentially explanatory and confounding factors. The fixed effects were treatment, lactation (as a surrogate for age and parity), DIM at first breeding, and DIM at conception or risk of conception. The model developed was extended to consider the potential impact of sire and the possibility of interval to conception being influenced through heredity by the animal’s sire and dam as well as the sire of conception. Two software functions, Cox mixed-effects and Cox proportional hazards, were used. The Kaplan-Meier (product limit) estimator was used to estimate the interval from parturition to conception for the survival analysis.

Nonsignificant variables and interaction terms identified during the initial modeling process were excluded from the final model. Thus, variables and interactions included in the final model were treatment, lactation, DIM at first breeding, and DIM at conception. Dependent variables consisted of days to conception or days to censoring and conception status. The explanatory variables treatment, lactation status, and DIM at conception were forcibly retained in all preliminary models and the final model. All models had the following general form:

\[ h_i(t) = h_0(t) \exp(\Sigma \beta_j X_j) \]

The hazard function, \( h_i(t) \), was the probability of the \( i \)th observation having a positive conception test result at \( t \) days after 44 DIM (initiation of risk period). The baseline hazard function, \( h_0(t) \), was the likelihood of conception at time \( t \), when all the independent (explanatory) variables were at their mean values. Unknown coefficients (\( \beta \)) were estimated for the \( X \) independent variables. Initial models used to examine the effect of the vaccine protocol on interval to conception included the variables for treatment, lactation number, DIM at first breeding, number of times bred, DIM at conception, season of first breeding, and season of conception were forcedly retained in all preliminary models and the final model. All models had the following general form:

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For evaluation of interval to conception, 4 categories of cattle were developed for time to event or censoring and coded separately as follows: inseminated but never conceived, inseminated and confirmed pregnant via manual or ultrasonic reproductive examinations, culled or died for unknown causes during the observation period and therefore did not receive a first insemination, or met all criteria throughout the observation period yet were never inseminated and therefore never conceived. To analyze the effect of lactation, cattle were grouped by lactation status as follows: heifer, first and second lactation, and third or greater lactation. Days in milk at first breeding was coded as 40 to 49, 50 to 59, 60 to 69, 70 to 79, or 80 to 128 days beginning at 44 DIM, which was recognized as the first day after parturition a female might be exposed to insemination. Because heifers had not yet given birth, a proxy measure for DIM at first breeding was assigned as 0 DIM (approx 11 to 13 months of age). Season of parturition, season of first breeding, and season of conception were coded as previously defined. Cattle that did not have a prior calving date (ie, heifers), first breeding date, or conception date were categorized collectively within their respective groups.

To estimate the conception rate, the cumulative proportion of pregnant cows and heifers was used. Logistic regression was used to estimate the proportion of heifers, first and second lactation cows, and third or later lactation cows within each treatment group that conceived during the observation period. Generalized linear modeling was used to determine the effect of the vaccine protocol and lactation status on the proportion conceiving.

The survival analysis technique used for the conception data was used to determine the interval from conception to pregnancy loss and to evaluate the effect of the vaccine protocol on failure to maintain a viable fetus. Logistic regression was used to estimate the proportion of heifers, first and second lactation cows, and third or later lactation cows within each treatment group that had a pregnancy loss. The proportion of pregnancy losses was used as a proxy measure for rate of pregnancy loss in each treatment and lactation group. A generalized linear model for binomial data was used to determine the effect of vaccine protocol and lactation status on the rate of pregnancy loss.

The Pearson \( \chi^2 \) test with Yates continuity correction was used to compare the initial herd prevalence (pretreatment) of Leptospira infection with the posttreatment prevalence, seroprevalence by lactation status at the initial and posttrial assessments, and initial seroprevalence with the posttrial seroprevalence in each treatment group. Values of \( P < 0.05 \) were considered significant for all analyses.

Results

Animals—Nine hundred eighty-six cattle comprised the vaccinated group, which included 325 (33.0%) heifers, 369 (37.4%) cows in their first or second lactations, and 292 (29.6%) cows in their third or greater lactation: 878 (89.0%) of vaccines became pregnant. The control group comprised of 908 cattle, which included 321 (32.0%) heifers, 330 (36.3%) cows in the first or second lactation, and 287 (31.6%) cows in their third or greater lactation; 804 (88.5%) of control cattle became pregnant.

Leptospirosis herd prevalence—Before the trial of the vaccine against L. borgpetersenii serovar Hardjo
strain hardjobovis began, 6 of the 46 (13%) cattle had positive results of testing for infection with the strain. Infected cattle included 2 of 23 (9%) nulliparous heifers, 3 of 21 (14%) first or second lactation cows, and 1 of 2 third or greater lactation cows. The prevalence of infection did not differ significantly ($P \geq 0.38$) among these lactation groups.

After the trial concluded, leptospires were detected in the urine samples of 6 of 40 (15%) cattle (vaccinated group, 3/24 [13%]; control group, 3/16 [18.8%]). Speciation by MAT confirmed the presence of antibody against *L. borgpetersenii* serovar Hardjo strain hardjobovis in all cattle with a positive urine test result. Comparison of the initial herd prevalence with the posttrial prevalence revealed no difference ($P \geq 0.93$), nor was a difference identified in prevalence when the initial prevalence was compared with that of the vaccinated ($P = 0.75$) and control ($P = 0.94$) groups after treatment. After treatment, 1 of 12 heifers, 4 of 18 first or second lactation cows, and 1 of 10 third or greater lactation cows were confirmed to be infected with strain hardjobovis, and the difference among these lactation groups was nonsignificant ($P \geq 0.61$). An equal proportion of vaccinated and control cattle had positive results for strain hardjobovis ($P \geq 0.98$), signifying no difference approximately 1 year after the trial commenced. A power calculation based on the assumptions of a 15% herd prevalence of *Leptospira* strain hardjobovis infection at the start of the trial and a vaccination protocol that would eliminate that infection revealed that the power to detect a difference between treatment groups was 20%.

**Effect of the vaccine protocol on pregnancy**—The probability of conceiving was not statistically different between the vaccinated and control groups, as indicated by a CI of the estimated HR of conceiving that included 1.0 ($95\% \text{ CI}, 0.88 \text{ to } 1.07; \text{ HR}, 0.97$ [Table 1; Figure 1]). No interactions among main effects were identified as significant. The mean number of days from the day a heifer or cow became eligible for insemination until conception was not significantly different between treatment groups. Eleven percent (108/986) of cattle in the vaccinated group failed to conceive, compared with 11.5% (104/908) in the control group.

The risk of heifers conceiving over the period at risk for conceiving, regardless of vaccination, was approximately 2.8 times as high as that for primiparous and pluriparous cows. During the study observation period, a similar cumulative proportion of vaccinated (96.6%) and control (96.5%) heifers became pregnant ($P > 0.05$). However, the cumulative proportion of pregnant heifers was greater than that of cows; the proportion of cows in their first or second lactation was similar between the vaccinated (86.7%) and control groups (86.3%), and these proportions were not significantly ($P \geq 0.05$) different from each other or from the proportion of cows in their third or later lactation (83.4% for both groups).

**Effect of DIM on conception**—Mean ± SE DIM at first insemination for primiparous and pluriparous cows was 61.7 ± 0.6 days and 61.2 ± 0.6 days for the vaccinated and control groups, respectively, suggesting that the groups had estrus at exactly the same time ($P > 0.05$). The proportion that conceived by 65 DIM or from first insemination (nulliparous heifers) was similar for vaccinated (43.0%) versus control (45.8%) cattle. The same was true for those that conceived by 128 DIM (76.4% vs 76.6% for vaccinated vs control cattle, respectively).

The mean DIM at conception was 105.5 ± 1.6 days for the vaccinated group and 101.4 ± 1.6 days, and these values were not significantly different. When the nonsignificant variable of vaccination status was removed from the regression model, the variable DIM at time of conception was significant ($P < 0.001$), suggesting cows were more likely to conceive at an earlier DIM than at a greater DIM (HR, 0.99 [Table 1; Figures 1 and 2]).

**Effect of lactation status on conception**—The mean number of lactations per cow in each group was not significantly different (vaccinated group, 1.8 ± 0.1 lactations; control group, 1.9 ± 0.1).In addition, the distribution of cows within the treatment groups was similar (HR, 0.95; 95% CI, 0.86 to 1.04; $P = 0.26$). The interval

![Figure 1](image-url)
from parturition to conception did not differ between first and second lactation cows and third or later lactation cows, nor did interactions of treatment by lactation number differ. Interval to conception was significantly different between heifers and cows in their first and second lactation or third and later lactation. Lactating cows had a longer interval from first breeding to conception than heifers and had a near equal risk of conception (HR, 0.35 for first or second lactation cows and 0.35 for third or later lactation cows; Table 1; Figure 2).

**Pregnancy loss**—One hundred forty-three cattle were recorded as having pregnancy loss during the study period. Of these, 74 were in the vaccinated group, representing 7.5% of all vaccinates and 8.4% of all vaccinates that became pregnant (n = 878), with a distribution of loss as follows: heifers, 2 of 314 (0.6%); first or second lactation cows, 36 of 320 (11.3%); and third lactation or greater cows, 36 of 244 (14.8%). The remaining 69 cattle with an aborted pregnancy in the control group (7.6% of all control cattle or 8.6% of all control cattle that became pregnant [804]) were distributed as follows: heifers, 0 of 281 (0%); first or second lactation cows, 37 of 285 (13.0%); and third lactation or greater cows, 32 of 238 (13.4%). Logistic regression revealed that the proportion of primiparous and pluriparous cattle that failed to maintain a conceptus was approximately equal between treatment groups. From the survival analysis, time from conception to pregnancy loss was not influenced by treatment (HR, 0.99; 95% CI, 0.70 to 1.38; \( P > 0.93 \)). Primiparous cows and cows with \( > 1 \) lactation were 3 times as likely to have pregnancy loss as were nulliparous heifers, but the HR was not significant (lactations 1 and 2, \( HR = 3.59 \) [95% CI, 0.57 to 22.60]; lactation 3 or greater, \( HR = 3.42 \) [95% CI, 0.55 to 21.50]). Only lactating cows had pregnancy loss after day 30 of gestation, with half of the losses (50.4%) detected in cattle in their first or second lactation, regardless of vaccination status.

**Discussion**

Infection with *L. borgpetersenii* serovar Hardjo strain hardjobovis may adversely affect the reproductive efficiency of North American dairy cattle, and an effective vaccine would be of economic importance. In the present study, a commercially available monovalent vaccine against *L. borgpetersenii* serovar Hardjo strain hardjobovis was administered in conjunction with long-acting oxytetracycline to Holstein dairy cattle naturally infected with strain hardjobovis as is recommended to control infection with that strain. Use of this vaccine protocol failed to significantly improve reproductive efficiency during the trial period. Interval from parturition to conception and conception rate were not significantly different between vaccinated and control cattle, indicating the vaccine protocol had no effect on fertility. The results are congruent with those obtained from another study in which effects were evaluated of the same vaccine protocol on reproductive performance in naturally infected beef cattle. In that study, as in ours, the vaccine protocol did not significantly improve pregnancy and calving rates.

Similar to the protocol in the other study, oxytetracycline was administered (20 mg/kg) to eliminate any existing or persistent *Leptospira* infections and to decrease transmission of leptospirosis among cattle in the herd. Use of antimicrobials in conjunction with initial vaccination was intended to initiate a primary antibody response against the pathogen and allow cattle to remain uninfected until the time of second vaccination between 28 and 35 days after first vaccination. In a trial similar to ours, although concurrent administration of oxytetracycline was not included in the vaccine protocol, a significantly increased rate of conception at first service (27% for control cattle and 36% for vaccinates) was achieved when 2 doses of the monovalent strain hardjobovis vaccine were administered. In the study...
Ruminants is linked to infertility and early embryonic death. Pre-plicated in 12% of bovine abortions in 1 UK study, losing a pregnancy at a greater DIM than at an earlier effect of the vaccine protocol on failure to maintain a previous research has shown seropositive test results for these serovars were consistent with the expected titers, and this information was taken into account when determining herd prevalence of *Leptospira* serovar Hardjo strain hardjobovis. The resulting titers were also expected because of the cross-reactivity of *Leptospira* serovars. An animal infected with 1 serovar of *Leptospira* will often produce antibodies against > 1 serovar as detected via MAT, which explains why some cattle that had positive results for strain hardjobovis also had measurable titers for other serovars. No cattle had positive results for any *Leptospira* serovar other than serovar Hardjo (strain hardjobovis).

Managing leptospirosis in dairy herds requires careful analysis of the problem and practical intervention protocols. An evaluation of cost of treatment and return on investment should be assessed prior to attempting any intervention. Antimicrobial treatment is believed to decrease the probability of renal and reproductive tract lesions indicative of persistent colonization by serovar Hardjo. The recommended protocol for the monovalent vaccine is an initial vaccination, in conjunction with administration of long-acting antimicrobials such as oxytetracycline, followed by a booster vaccination 4 to 6 weeks later. We estimated the cost of implementing the vaccine protocol to be $7.20/cow/y ($4 for 2 doses of vaccine and $3.20 for 1 dose of oxytetracycline). An annual vaccine booster is also recommended. Cumulatively, the expense of the suggested *Leptospira* vaccine protocol for a 2,000–milk cow dairy, with 1,000 heifers entering the herd each year, would be approximately $20,000 for the first year.

Our study had limitations, and possible reasons existed to explain why the vaccine protocol did not lead to successful improvement of reproductive performance in the study herd. Throughout the study, vaccinated cattle were housed with control cattle, which might have resulted in a herd-protective immunization effect from the vaccines. We believe this to have been possible because use of the vaccine can result in a decrease in urinary shedding of spirochetes by infected cattle and antimicrobial administration can also stop...
and fluids at the time of pregnancy loss might have infected the vaccinated animals had the antimicrobial worked successfully. In addition, because of the possible vertical transmission from the dam to the fetus, diagnostic testing of fetal serum, kidney tissue, and fluids at the time of pregnancy loss might have yielded insight as to the cause of pregnancy loss.13

The lack of treatment effectiveness, whether ascribed to vaccination or antimicrobial use, suggested that current methods of controlling serovar Hardjo strain hardjobovis in Holstein dairy cattle in conditions typical for commercial milk production in California is ineffective. We acknowledge that although there was no significant difference between the study treatment groups, the sample size was too small to discern minor differences in prevalence. However, had the vaccine and antimicrobial protocol been effective, the treated group would have had no infected cattle and there would have been marked differences in interval to conception in the treated versus control group, which was not observed.

Another limitation is few apparent clinical consequences of Leptospira infection were observed in the study herd; some common clinical features not observed include pyrexia, septicemia, and jaundice.14 The literature regarding clinical infection due to serovar Hardjo in dairy cattle provides conflicting information. The clinical signs of serovar Hardjo infection can range from absent to mild, but infected dairy cattle typically have a decrease in milk production and reproductive efficiency.10 Few clinical signs of infection were expected because of the ability of host-adapted serovars (ie, serovar Hardjo) to maintain persistent infection in the host, whereas other serovars cause sporadic infections43 with more prominent clinical signs. Clinical evidence of disease was not reported for other Leptospira vaccine efficacy studies4,5,33 despite bacterial and serologic evidence of infection.

The observation that breeding efficiency in a dairy herd with endemic leptospirosis was not improved after inoculation with the monovalent vaccine against Leptospira borgdetersenii serovar Hardjo strain hardjobovis provides evidence that this protocol was not effective when administered to naturally infected dairy cattle. By comparing the results of the previous studies3,4,13 with ours, we concluded that administration of this monovalent vaccine with oxytetracycline was unable to improve reproductive efficiency in naturally infected Holstein dairy cows and heifers. Our findings conflict with those of another study22 in which administration of the same vaccine effectively eliminated urinary shedding. However, the results were also congruent with those of other research involving beef cattle,1 suggesting the vaccine protocol was not successful when administered as a treatment and prevention technique in 2 types of cattle production systems. We recommend that cattle producers and bovine practitioners carefully evaluate the potential costs and benefits prior to implementing a protocol that involves the evaluated monovalent vaccine.

References


