



PROFESSIONAL SERVICES

Boehringer Ingelheim Vetmedica, Inc.

TECHNICAL BULLETIN

Protection for *Lepto borgpetersenii* serovar *hardjo* using Express® FP

RESEARCH CONDUCTED BY

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OVERVIEW

New indications for Express FP include:

- **Prevention of urinary shedding of *Leptospira borgpetersenii* serovar *hardjo* (type *hardjo-bovis*) and,**
- **Vaccinated animals subsequently exposed to *L. borgpetersenii* serovar *hardjo* (type *hardjo-bovis*) have been shown to clear renal infections within 8 weeks of exposure.**

These represent major advancements for the Lepto 5 component of Express FP. In this study, twenty-one heifers vaccinated with Express FP were 100% protected against urinary shedding of “*hardjo-bovis*” on days 7, 14, 21, 28, 35, 42, 49 and 55 post-challenge. All placebo control heifers had positive urine cultures from a minimum of two post-challenge samples. No animals in the vaccine group had leptospire cultures from kidney tissues, while 10 of 11 heifers in the control group had positive cultures. Post-challenge serological microagglutination titer (MAT) response to *L. hardjo* challenge was positive for all challenge animals, yet all vaccinated animals remained MAT negative post-challenge, suggesting that the immune system of the vaccinated heifers, including circulating lymphocytes, were not exposed to a sufficient pathogen load to give an anamnestic serological immune response. The implication is that Express FP provides veterinarians and producers a new option for **effective** protection against *L. hardjo*.

INTRODUCTION

The purpose of this study was to prove that Express FP provides effective protection against a virulent challenge of *Lepto hardjo*. *Leptospira interrogans* serovar *hardjo* (type *hardjo-prajitno*) (LHP) is a European serotype which is included in most domestic 5-way Lepto vaccines. However, *Leptospira borgpetersenii* serovar *hardjo* (type *hardjo-bovis*) (LHB) is the serotype that is found worldwide and is the only type of serovar *hardjo* isolated from cattle in the United States.^{1,2} It has long been held that traditional Lepto 5 vaccines containing LHP did not provide cross protection for LHB.^{3,4} LHB is a recognized pathogen in cattle and has been associated with early embryonic deaths (EED), poor conception rates, weak calves, and other reproductive losses in cattle and other domestic species.⁵ In addition, it is a recognized public health concern.^{6,7} Solid protection against LHB with a vaccine containing a traditional *Leptospira interrogans* serovar type *hardjo* component of Lepto 5 bacterin offers veterinarians and cattle producers convenient and cost-saving options in lieu of vaccines containing LHB antigen. Therefore, Boehringer Ingelheim Vetmedica, Inc., conducted a study to prove cross-protection of LHB. The primary parameters used to evaluate the efficacy of the LHP vaccine included:

- Significant reduction of renal infection/colonization in vaccinated animals compared to placebo controls based on recovery of the challenge organism from kidney tissue.
- Significant reduction of urinary shedding of the challenge strain of *Leptospira* in vaccinated animals compared to placebo controls based on recovery of the challenge organism from urine samples.

MATERIALS AND METHODS

Animals. Ten days prior to vaccination, thirty-four (34) six-month-old Holstein heifers arrived at a dry lot housing facility located near Brookings, SD. All animals had no vaccination history to *Leptospira* and were serologically negative to six serovars of *Leptospira* (*pomona*, *hardjo*, *grippotyphosa*, *bratastava*, *canicola*, and *icterohaemorrhagiae*) by *Leptospira* Microscopic Agglutination Titer test (MAT).[†] All animals were negative to Bovine Viral Diarrhea Virus (BVDV) via immunohistochemical (IHC) testing. Thirty-two (32) animals were included in the study and the additional two (2) animals were retained for the passage of the challenge material.

Animal Enrollment, Randomization and Animal Observations. Animals were assigned to treatment groups following a completely randomized distribution using the Random Numbers Generator in the Analysis ToolPak provided by Microsoft® Excel. The investigator and/or designees were blinded to the individual assignments to treatment groups. Animals were commingled and housed in a dry lot with a concrete bunk and automatic waterers.

Treatments. The following viral/bacterin combination vaccines were used in the study:

Treatment Group 1: Vaccinates were administered a modified-live combination product consisting of: Bovine Rhinotracheitis (IBR), Virus Diarrhea (BVDV Types I & II), Parainfluenza₃ (PI₃), Respiratory Syncytial Virus Vaccine (BRSV), modified-live virus (MLV) fraction plus *Campylobacter fetus*, *Leptospira canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, and *L. pomona* bacterin (Licensed combination product: Express® FP 5-VL5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO).

Treatment Group 2: The placebo vaccinated control group was administered a modified live virus product consisting of IBR, BVD, PI₃, BRSV (Licensed combination product: Express® FP 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO)

Group 3 Untreated Controls: Two animals were used for production of challenge inoculum.

The products used for vaccination were fully released commercially available products. All vaccinations were administered via the subcutaneous (SC) route. The test vaccine and placebo were administered as two doses, each 2 mL as indicated in Table 1, with a 21-day interval between the vaccinations.

Table 1 Treatment Groups, Number of Animals and Vaccine Dose Administered

Treatment Group	Number of Animals	Treatment/Administration	Dose
1	21	Express FP 5-VL5 (Test Group)/Subcutaneous	2 x 2 mL
2	11	Express FP 5 (Placebo Controls)/Subcutaneous	2 x 2 mL
3	2	Challenge Passage/Intraocular	N/A

Serology: Blood samples were collected from all animals on post-vaccination (PV)[‡] days 0, 21, 35, 43, 105, 112, 119, 126, 133, 140, 147, 154 and 160 via jugular venipuncture. Serum samples were submitted to South Dakota State University Animal Disease Research and Diagnostic Laboratory (SDSU ADRDL) for *Leptospira* MAT testing to determine antibody response to *Leptospira canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae* and *pomona*. Based on the SDSU ADRDL MAT procedure, a MAT endpoint titer >100 was considered to be positive. Strain LHP was used in the *L. hardjo* MAT.

Urine Culture: Urine was collected from each heifer on PV days 0, 105, 112, 119, 126, 133, 140, 147, 154, and 160. Samples were collected during mid-stream urination. The samples were obtained after administering 500 mg furosemide (Vedco, Phoenix Scientific, Inc., St. Joseph, MO) intravenously (IV) to each heifer. Samples were inoculated in Ellis (80/40) culture medium and incubated at 29°C for up to two months. Cultures were observed microscopically weekly for signs of *Leptospira* growth. Culture samples showing positive growth were confirmed to be *Leptospira* by dark-field microscopy. Cultures showing no growth after 2 months of observation were considered to be negative.

Kidney Cultures: On PV day 161 and 162 (56 and 57 days post challenge [DPC]), animals were transported to SDSU ADRDL for necropsy and tissue sample collection. Immediately following euthanasia, both kidneys were removed. If suspect areas of the kidney were noted, characterized by depressions or white areas, tissue samples totaling approximately 1 cm²/1 gram of tissue per kidney were obtained from those areas. If no suspect areas were seen, samples were taken 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 two months. Cultures were observed microscopically weekly for signs of *Leptospira* growth. Culture samples showing positive growth were confirmed to be *Leptospira* by dark-field microscopy. Cultures showing no growth after 2 months of observation were considered to be negative.

Challenge: *Leptospira borgpetersenii hardjo bovis* strain 203 was used to challenge study animals following the two-animal challenge passage phase. On days 105, 106, and 107 PV, 1 mL of challenge material containing approximately 10⁶ leptospire per mL was used to challenge the heifers intraocularly. (E.g. 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, one eye at a time. Each eyelid was held closed for 60 seconds following administration of the challenge material.)

RESULTS

Microagglutination Titer Test Results: Blood samples were taken from all enrolled heifers in Groups 1, 2 and 3 on PV days 0, 21, 35 and 43 for MAT testing to determine antibody response to *Leptospira canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae* and *pomona*. Transient post-vaccination MAT serological responses were detected to the *L. canicola*, *grippotyphosa*, *icterohaemorrhagiae* and *pomona* serovars in 40 to 75 percent of the vaccinated heifers on 14 and 21 days after the second vaccination in both vaccination groups. Positive MAT serological response to the *L. hardjo* was observed only on day 14 post second vaccination in less than 24 percent of the vaccinates. All unvaccinated controls remained negative to all serovars through the day of challenge.

Post-challenge blood samples were taken from all enrolled heifers on DPC 0, 7, 14, 21, 28, 35, 42, 49 and 55 for MAT testing to determine antibody response to *Leptospira canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae* and *pomona*. Post-challenge MAT antibody responses remained negative for *L. canicola*, *grippotyphosa*, *icterohaemorrhagiae* and *pomona* through the end of the study. Post-challenge MAT results for the *L. hardjo* remained negative in all vaccinates through the end of the study. However, post-challenge MAT antibody response was evident in 9 of 11 Group 2 controls by day 14 post-challenge, and in 11 of 11 Group 2 controls by day 21 post challenge. Peak MAT titers in the unvaccinated controls ranged from 800 to 1600.

Urine Shedding Results: All pre- and post-challenge urine cultures from all urine samples in the vaccinates were negative for growth of leptospire ($p < 0.0001$). All placebo control Group 2 heifers had positive urine cultures from a minimum of two post-challenge samples. (Table 2) The calculated prevented fraction for urine culture sample results for both vaccine groups compared to the control was 1.0000 (100%) and the corresponding exact 95% confidence interval was [0.9389, 1.0000] or [83.5%, 100%]. No heifers in either vaccine group had any positive urine culture results and 11 of 11 heifers in the control group had positive urine culture results.

Table 2 Urine Culture Results

Group	# of Heifers Positive	# of Heifers Negative	Percent Positive
1	0	21	0%
2	11	0	100%

Kidney Culture Results: The results of cultures on kidney tissues from Groups 1 and 2 are summarized in Table 3. There were no leptospire isolations from the kidneys of any vaccinated heifer, while 10 of 11 heifers in the control group had positive leptospire cultures from kidney tissue.

Table 3 Kidney Culture Results

Group	# of Heifers Positive	# of Heifers Negative	Percent Positive
1	0	21	0%
2	10	1	90.9%

The calculated prevented fraction for kidney tissue culture sample results for both vaccine groups compared to the control was 1.0000 (100%) and the corresponding exact 95% confidence interval was [0.8349, 1.0000] or [83.5%, 100%]. No heifers in the vaccine group had any positive culture results and 10 of 11 heifers in the control group had positive kidney culture results.

Prevented fractions and 95% confidence limits for the urine shedding and kidney culture results are provided in Table 4.

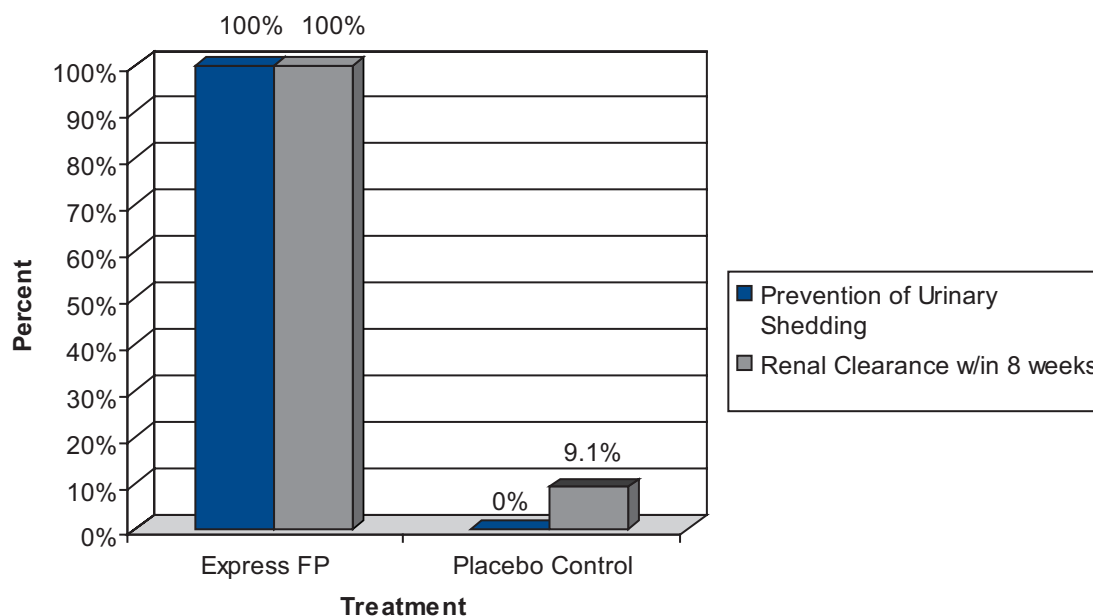
Table 4 Prevented Fraction and Exact 95% Confidence Limits for Presence of Organism Recovery

Analysis Variable	Treatment Group	Prevented Fraction	Exact P-value	Lower 95% CL	Upper 95% CL
Urine	1 vs. 2	1.0000	< 0.0001	0.8389	1.0000
Kidney	1 vs. 2	1.0000	< 0.0001	0.8349	1.0000

DISCUSSION

Vaccination with a modified live viral *Leptospira interrogans* serovar *hardjo* (type *hardjo-prajitno*) bacterin combination resulted in prevention of urinary shedding following challenge. In addition, vaccinated animals that subsequently were challenged with *Leptospira borgpetersenii* serovar *hardjo* (type *hardjo-bovis*) were shown to have cleared the renal infection within eight weeks. The results of this study show that the *Leptospira hardjo* component of the Boehringer Ingelheim Vetmedica, Inc. vaccines is highly efficacious against challenge-induced renal infection and colonization and associated urinary shedding due to *Leptospira borgpetersenii hardjo-bovis*.

Figure 1 *Lepto hardjo* Control



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† Testing performed by South Dakota State University Animal Disease Research and Diagnostic Laboratory. Serovars tested included *Leptospira canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae* and *pomona*. *Leptospira* serovar *hardjo* (type *hardjo-prajitno*) was used for the L *hardjo* MAT.

‡ Post-vaccination (PV) refers to days following the vaccination.

BIVI Study: 6128-1301-06B-164. C. Rinehart, C. Chase, A. Zimmerman. Efficacy of vaccination of cattle with the *Leptospira interrogans* serovar type *hardjo* component of Lepto 5 Bacterin against challenge with *borgpetersenii* serovar type *hardjo-bovis*.

