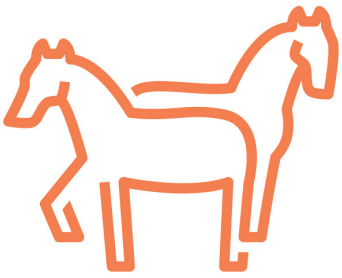
zoetis

# TECHNICAL BULLETIN

October 2015



## Introducing LEPTO EQ INNOVATOR® for Aiding in the Prevention of Leptospirosis Caused by *Leptospira interrogans* Serovar Pomona in Horses

**Jacquelin Boggs, DVM, MS, ACVIM**
**Senior Veterinarian**
**Equine Technical Services**
**Zoetis**

### Key Points

- Leptospirosis in horses, a difficult disease to recognize and diagnose, is responsible for serious and costly health problems, including acute renal failure, abortion in pregnant mares, and equine recurrent uveitis (ERU) that can result in blindness.[1,2]
- *Leptospira interrogans* serovar Pomona, acquired from wildlife and domestic host species, is the leptospiral serovar most frequently associated with clinical disease in horses in North America.[1,3]
- Control of leptospirosis has historically relied upon biosecurity measures including minimizing exposure to wildlife and other domestic species capable of shedding leptospires into the environment as well as avoiding exposure to standing water.
- The introduction of LEPTO EQ INNOVATOR®, a whole-cell monovalent *Leptospira* bacterin with a label claim for use as an aid in prevention of leptospirosis caused by *Leptospira interrogans* serovar Pomona, marks the advent of a new era in the control of leptospirosis in horses.
- Species- and serovar-specific, LEPTO EQ INNOVATOR is formulated with MetaStim®, a proprietary adjuvant system designed for safe, enhanced presentation of *L. pomona* antigens to the horse's immune system.
- Immunogenicity of LEPTO EQ INNOVATOR was demonstrated in a vaccination and challenge study conducted with 6-month-old horses. Field safety was demonstrated in foals 3 months of age or older.

# EQUINE LEPTOSPIROSIS:

# Difficult to Detect, Serious and Costly to Miss

## Scope of the Problem

A disease of worldwide distribution and zoonotic potential, equine leptospirosis is defined as an acute bacterial infection of horses caused by pathogenic spirochetes belonging to the genus *Leptospira*.[1,4] Leptospires can infect the horse through abraded or soft, moist skin and mucous membranes and can colonize in the kidney and be shed in the urine. As with other infectious diseases, the outcome of exposure to *Leptospira* depends on the dose, virulence of the infecting serovar, and host susceptibility. Leptospirosis generally follows a biphasic course, with a leptospiremic phase lasting about 8 days, followed by an immune phase during which antibody is produced and leptospires are shed in the urine.[5] The invasion of internal organs occurs during the leptospiremic phase, with severity of the infection depending upon the presence of a humoral immune response or the ability and speed of the horse to mount a response. If the antibody response is inadequate, severe tissue damage may result.[6] Typically, leptospirosis in horses is subclinical. Only occasionally do *Leptospira* cause fever and acute renal failure, typically shown in young horses.[7-9] Kidneys become swollen due to tubulointerstitial nephritis, and the urine may have pyuria without visible bacteria.[1] Infected horses may shed pathogenic leptospires in their urine for up to 5 months and can potentially transmit the disease to horses they come into contact with.[10] Often, horses are only mildly affected (listless with a low-grade fever) initially; thus, a diagnosis of leptospiral infection is often missed.[2] In addition, *L. pomona* has been associated with two pathological conditions in North American horses: uterine infections resulting in abortions in mares and recurrent uveitis potentially leading to blindness in horses of all ages.[1,5-7]

Currently, there are no vaccines available with USDA licensed label claims against abortions, recurrent uveitis, or acute renal failure caused by *L. pomona*.

Until the introduction of LEPTO EQ INNOVATOR, no equine vaccine for the prevention of leptospirosis has been commercially available for use in horses. Prevention has focused on good husbandry and hygiene practices, vaccination of cattle, swine, and dogs on the same farm, and minimizing contact with wildlife carriers, and other infected horses.[1,10.11] The practicality of implementing biosecurity measures such as providing clean, uncontaminated drinking water, fencing off access to potentially contaminated ponds and streams, controlling rodent populations, and separating horses from cattle, pigs, and wildlife or infected domestic species may vary considerably from operation to operation, and can be challenging.

## Rationale for LEPTO EQ INNOVATOR®

LEPTO EQ INNOVATOR was introduced in the U.S. by Zoetis in 2015 to address the needs of equine veterinarians and horse owners. A killed whole-cell vaccine was formulated with an equine isolate of *L. pomona* and the proprietary MetaStim® adjuvant system. In North America, *L. pomona* is the most common cause of clinical disease in horses.[1,3,7] Based on the literature and input from leading equine leptospiral researchers indicating that *L. pomona* posed the most severe and most immediate threat to the equine industry in the U.S.,[1,3,7,12–14] Zoetis developed LEPTO EQ INNOVATOR as a monovalent species-specific vaccine. LEPTO EQ INNOVATOR is the only leptospiral vaccine marketed that helps protect horses against bacteremia, uremia, and kidney colonization caused by *L. pomona*.[15]

## Vaccine Composition: The MetaStim® Advantage

LEPTO EQ INNOVATOR is produced using an equine isolate of *L. pomona* that undergoes an intensive purification process to produce a whole-cell vaccine with low reactivity. The *L. pomona* vaccine antigen is micro-filtered a total of five times by diafiltration, a process that helps remove extraneous protein from the vaccine's antigen components, resulting in a vaccine with purity and smoothness.

Immunogenicity of the *L. pomona* antigens is potentiated by the addition of the MetaStim adjuvant system. MetaStim is a dual-phase formulation system consisting of lipid droplets in an aqueous surfactant solution. Equine vaccines adjuvanted with MetaStim have been shown to stimulate fast, antigen-specific, serological and cellular immune responses.[16-18]

## Product Efficacy and Safety[15,19]

Scientists at the Zoetis research facility located in Kalamazoo, Michigan developed a challenge model to demonstrate that LEPTO EQ INNOVATOR aids in the prevention of leptospirosis caused by *L. pomona*. This challenge model demonstrated that challenge with *L. pomona* organisms would result in leptospiremia, leptospiral colonization of equine kidneys, and urinary shedding of leptospires by horses.[19] Furthermore, safety was evaluated by monitoring horses for adverse reactions following vaccination.

### Protocol

A total of thirty 6-month-old horses, seronegative (microscopic agglutination titer (MAT) < 8 to *L. pomona, L. bratislava, L. icterohaemorrhagiae, L. grippotyphosa, L. hardjo,* and *L. canicola*) were enrolled in the study. Fifteen horses served as saline-vaccinated controls and 15 received LEPTO EQ INNOVATOR®. All horses received two doses of the respective test articles, administered intramuscularly 3 weeks apart. Starting 21 days following the second vaccination, all horses were challenged with virulent *L. pomona* organisms on 3 consecutive days (Days 42–44) by the intraperitoneal route, a route of challenge established in previous studies.[20] Horses were monitored daily for clinical signs of disease beginning on Day 40 (2 days prior to challenge) through Day 56 and on Days 63 and 70. Blood and urine samples were cultured and examined for *L. pomona*. Four weeks after challenge, all horses were humanely euthanized (Day 70 or 71), and kidney and liver tissues were harvested and examined for evidence of leptospiral colonization. The re-isolation of *L. pomona* from urine, blood, kidney, and/or liver samples from control horses demonstrated colonization and infection.

The safety assessment consisted of calculating frequency distributions of injection site observations (reaction, heat, pain, and swelling) for each vaccination group and each vaccination time point.

The entire study was conducted in compliance with applicable corporate and governmental animal welfare guidelines and regulations.

### Results

MAT serology showed all 30 test horses were seronegative to *L. pomona, L. bratislava, L. icterohaemorrhagiae, L. grippotyphosa, L. hardjo,* and *L. canicola* prior to vaccination. All control horses remained negative until after challenge, whereas horses vaccinated with LEPTO EQ INNOVATOR developed antibody titers following vaccination. The mean post-challenge *L. pomona* antibody titers of horses in the LEPTO EQ INNOVATOR test group increased less than two-fold, indicating that infection was limited. Post-challenge clinical signs were unremarkable in all horses. Virulent challenge was demonstrated in that 10 of

15 horses in the control group, vaccinated with saline, developed fevers ≥ 103.0°F as compared with only 3 horses vaccinated with LEPTO EQ INNOVATOR (104.0°F, 103.6°F, 103.0°F) (**Table 1**). *L. pomona* was isolated from all 15 control group horses. In comparison, leptospires were not detected in the urine, blood, or kidney samples of any of the horses vaccinated with LEPTO EQ INNOVATOR. Thus, the LEPTO EQ INNOVATOR preventable fraction for *L. pomona* isolation was 100% (95% CI: 78%, 100%). *L. pomona* was not isolated from any liver samples in either test group.

### Conclusion

The challenge study documented the efficacy and safety of monovalent LEPTO EQ INNOVATOR. Vaccination with LEPTO EQ INNOVATOR prevented leptospiremia, kidney colonization, and urinary shedding following challenge with *L. pomona*. In identically challenged control group horses, leptospiremia (15/15), urinary shedding (14/15), and kidney colonization (3/15) were observed. Safety of LEPTO EQ INNOVATOR® was also demonstrated as no abnormal post-vaccination reactions and no adverse events were attributed to administration of the vaccine. Collectively, the results supported the vaccination of healthy horses, 6 months of age or older, as an aid in the prevention of leptospirosis caused by *L. pomona*.

**Table 1.**
**Results of examination of blood, urine, and kidney samples from horses vaccinated with LEPTO EQ INNOVATOR® following IP challenge with virulent *L. pomona*[15]**

| Assessment | Controls (n = 15) | Vaccinates (n = 15) |
|---|---|---|
| Fever* | 10 | 3 |
| Blood isolation | 15 | 0 |
| Urine isolation | 14 | 0 |
| Kidney isolation | 3 | 0 |
| **Total positive isolation (%)** | **100** | **0** |

IP = intraperitoneal *Fever defined as temperatures ≥ 103.0° F

### Benefit

By preventing *L. pomona* colonization of the kidney, LEPTO EQ INNOVATOR helps stop shedding of infectious *L. pomona* in urine, therein helping minimize contamination of the environment, transmission to other horses on the farm, and development of carrier animals. Because horses excreting *L. pomona* may play a role in disease transmission,[10] one of the keys to controlling equine leptospirosis is the prevention of renal tract colonization, which stops leptospirosis before it gets started. By administering LEPTO EQ INNOVATOR, owners and their veterinarians have the additional peace of mind that comes with knowing that vaccinated horses are protected against *L. pomona*.

## Field Safety[21,22]

The field safety of LEPTO EQ INNOVATOR was evaluated in two phases in two different groups of horses:

- **Phase 1:** Healthy horses (n = 681) in two age categories (foals 3 months of age or younger and horses 5 months of age or older) and
- **Phase 2:** Pregnant mares (n = 338) of various breeds in all three trimesters of pregnancy (1st trimester = 13; 2nd trimester = 298; and 3rd trimester = 27) including controls.

### Phase 1 Protocol[21]

Of the 681 animals representing 10 different breeds from five different geographic regions, 207 were foals ≤ 3 months of age (age range: 2 to 3 months) and 474 were horses ≥ 5 months of age (age range: 5 months to 21 years). All horses were sourced from commercial farms or private owners, were randomly allotted to one of two treatment groups, and were administered 2 doses of either one of two pre-licensing serials of LEPTO

EQ INNOVATOR. On Study Day 0 and again on Study Day 21, all horses in group T1 (n = 340) were vaccinated intramuscularly with vaccine from one serial and all horses in group T2 (n = 342) with vaccine from the second serial. Following each vaccination, all animals were observed for at least 20 to 30 minutes for signs of local or systemic reactions. Beginning on Day 1 of the first vaccination through 21 days after the second vaccination, all animals were observed at least once daily for general health (injection site reactions, fever, inappetence, nasal and ocular discharge, lameness, abdominal pain, loss of condition, edema).

## Phase 1 Results

During the course of the study, a total of 1,358 vaccinations were administered. No immediate or systemic reactions were observed in any animals within 20 to 30 minutes after either the first or second vaccination. Injection site reactions were observed in 3 of 681 animals (T1: n = 1; T2: n = 2). All 3 injection site reactions had resolved or were resolving by the end of the study. Abnormal health events occurring during the study—other than the 3 injection site reactions—were unrelated to administration of the vaccine and had an association with the type, age, and management of the animals on sites where the study was conducted. The number of injection site reactions was 3 of 681 animals (0.004%) and 3 of 1,358 vaccinations (0.002%), demonstrating the safety and low reaction rate of LEPTO EQ INNOVATOR.

## Phase 2 Protocol[22]

The 338 healthy pregnant mares representing 13 different breeds from three geographically separate regions enrolled in the second phase of the field safety study were randomly allotted to one of three treatment groups, with horses in T1 (n = 112) and T2 (n = 113) receiving two doses of one of two pre-licensing serials and horses in T3 (n = 113) receiving two doses of a saline control product. Of the 338 mares, 13 were in the first trimester of pregnancy; 298 were in the second trimester; and 27 were in the third trimester. On Day 0 and again on Day 19 to 29, 1 mL of the appropriate vaccine or saline control product was administered intramuscularly. All animals were observed for at least 30 minutes after each vaccination for any local or systemic reactions. Beginning on Day 1 of the first vaccination through 21 days after the second vaccination, all animals were observed at least once daily for general health. Additionally, on or a few days prior to Day 0 and again on the last day of the study, all pregnant animals were checked for pregnancy status by palpation, ultrasound, or serology test (estrone sulfate).

## Phase 2 Results

Of the 338 animals enrolled, 328 (97%) completed the study. After administration of the first vaccine dose, one owner removed 10 horses (all in the second trimester) on account of a change in residency. Remaining in the study were 13 mares in the first trimester, 288 in the second trimester, and 27 in the third trimester. In total, 665 vaccinations were administered, 221 to horses in the T1 group, 222 to horses in the T2 group, and 222 to horses in the saline control group. There were no immediate systemic or local reactions associated with administration of either investigational vaccine or the saline control product. Additionally, no animals were removed from the study due to abnormal health events or animal welfare issues.

There were two mares that aborted; however, no fetus was found, which precluded a definitive diagnosis of the cause of abortion. Paired serum samples, a uterine swab, and a uterine biopsy sample were collected from each mare that aborted in an attempt to obtain diagnostic data; however, a definitive cause was not identified. The overall low

rate of abortion in the study (n = 2/288, 0.69% of horses vaccinated during the second trimester) was well below the reported 6% to 10% natural rate of abortion in mares greater than 40 days of gestation,[23,24] and was not indicative of a vaccine safety issue.

## Field Safety Study Conclusions

In both phases of the field safety study, no significant adverse reactions to vaccination with LEPTO EQ INNOVATOR were observed. Overall conclusion of phase 1 of the field safety studies was that the vaccine is safe for use in horses 3 months of age or older. A similar conclusion was reached in the second phase of the field safety studies for mares in the second trimester of pregnancy. Based upon the type and rate of abnormal events recorded, the investigator's abnormal health event documents, and diagnostic laboratory reports, the conclusion was that the observed abnormal health events were not related to administration of the vaccine. Thus, the data generated from the second phase of the study demonstrated the safety of LEPTO EQ INNOVATOR in pregnant horses in the second trimester under field use conditions. Safety in the first and third trimesters could not be evaluated due to the low number of horses in each group.

## Benefit

Because LEPTO EQ INNOVATOR can be safely administered to horses 6 months of age or older, vaccination can help prevent kidney colonization and urinary shedding, which means that LEPTO EQ INNOVATOR can help prevent contamination of the environment and transmission to other horses, as well as helping provide protection to horses exposed to leptospiral organisms in the environment, factors considered key to controlling leptospirosis outbreaks caused by *L. pomona*.

# SUMMARY

Leptospirosis in horses is likely an underdiagnosed disease that is capable of causing substantial economic and emotional damage to horse owners and the horse industry. In U.S. horses, the leptospiral agent most often associated with pathological conditions is *L. pomona*.[1,3,7] Control efforts have focused on good husbandry and hygiene practices directed at limiting direct or indirect contact with the urine of potential carriers such as cattle, swine, striped skunk, raccoon, white tail deer, and opossum, minimizing exposure to urine or fetal tissues of infected horses, and limiting exposure to stagnant or standing water. The 2015 introduction of LEPTO EQ INNOVATOR® marks the beginning of a new approach to the control of equine leptospirosis caused by *L. pomona*. A challenge-of-immunity study was conducted that supports the label claim of aiding in the prevention of leptospirosis caused by *L. pomona* in horses 6 months of age or older. Vaccination with MetaStim-adjuvanted LEPTO EQ INNOVATOR stimulated a serological response that helped protect vaccinated horses against *L. pomona* challenge. No challenge leptospires were detected in blood, urine, or kidney samples from any of the 15 horses (0%) in the LEPTO EQ INNOVATOR vaccination group. However, leptospires were isolated from one or more tissue/ fluid samples from all 15 (100%) of the saline vaccinated horses. LEPTO EQ INNOVATOR was also shown to be a safe vaccine as only 3 self-resolving injection site reactions were observed following administration of 1,358 doses to horses, some as young as 2 months of age, including 288 doses in pregnant mares in the second trimester. LEPTO EQ INNOVATOR was shown to be 99.8% reaction-free across the vaccinated horses. For horse owners and veterinarians alike, LEPTO EQ INNOVATOR provides a new tool in the battle against potential losses associated with a *L. pomona* infection that often goes unrecognized until its damage has been done. Vaccination can help prevent kidney colonization and urinary shedding, which means LEPTO EQ INNOVATOR can help prevent contamination of the environment and transmission to other horses, important factors to consider in controlling leptospirosis outbreaks caused by *L. pomona*.

# REFERENCES


1. Divers TJ, Chang Y-F. Leptospirosis. In: Robinson NE, Sprayberry KA, eds. *Current Therapy in Equine Medicine*. Vol 6. 6th ed. St. Louis, Mo: Saunders Elsevier;2009:145–147.

2. Thomas H. Leptospirosis in horses. *Equine Chronicle*. Available at: www.equinechronicle.com/leptospirosis-in-horses. Accessed June 23, 2015.

3. Spirochaetis. In: Quinn RJ, Markey BK, Leonard FC, Fitzpatrick ES, Fanning S, Hartigan PJ, eds. *Veterinary Microbiology and Microbial Disease*. 2nd ed. Ames, Iowa. Wiley-Blackwell; 2011:354–367.

4. Bharti AR, Nally JE, Ricaldi JN, et al. Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis* 2003;3(12):757–771.

5. Hines MT. Leptospirosis. In: Sellon DC, Long MT, eds. *Equine Infectious Diseases*. St. Louis, Mo. Saunders Elsevier:2007:301–309.

6. Bernard WV. Leptospirosis. *Vet Clin North Am* 1993;9(2):435–444.

7. Divers TJ. Equine leptospirosis (Proceedings). *DVM360*. Available at: http://veterinarycalendar.dvm360.com/equine-leptospirosis-proceedings. Accessed July 6, 2015.

8. Divers TJ, Byars TD, Shin SJ. Renal dysfunction associated with infection of Leptospira interrogans in a horse. *J Am Vet Med Assoc* 1992;201(9):1391–1392.

9. Frellstedt L, Slovis NM. Acute renal disease from Leptospira interrogans in three yearlings from the same farm. *Equine Vet Educ* 2009;21(9):478–484.

10. Frellstedt L. Equine recurrent uveitis: a clinical manifestation of leptospirosis. *Equine Vet Educ* 2009;21(10):546–552.

11. Verma A, Stevenson B, Adler B. Leptospirosis in horses. *Vet Microbiol* 2013;167:61–66.

12. Timoney JF, Kalimuthusamy N, Velineni S, et al. A unique genotype of *Leptospira interrogans* serovar Pomona type kennewicki is associated with equine abortion. *Vet Micro* 2011;150: 349–353.

13. Erol E, Jackson CB, Steinman M, et al. A diagnostic evaluation of real-time PCR, fluorescent antibody and microscopic agglutination tests in cases of equine leptospiral abortion. *Equine Vet J* 2015;47:171–174.

14. Yan W, Faisal SM, Divers T, et al. Experimental Leptospiral interrogans Serovar Kennewicki infection of horses. *J Vet Intern Med* 2010;24:912–917.

15. Data on file, Study Report No. B850R-US-12-011, Zoetis Inc.

16. Spickler AR, Roth JA. Adjuvants in veterinary vaccines: modes of action and adverse effects. *J Vet Intern Med* 2005;17:273–281.

17. Davis EG, Zhang Y, Tuttle J, et al. Investigation of antigen specific lymphocyte responses in healthy horses vaccinated with an inactivated West Nile virus vaccine. *Vet Immunol Immunopathol* 2008;126(3–4):293–301.

18. Horohov DW, Dunham J, Liu C, et al. Characterization of the *in situ* immunological responses to vaccine adjuvants. *Vet Immunol Immunopathol* 2015;164:24–29.

19. Data on file, Study Report No. 3151W-60-12-177, Zoetis Inc.

20. Yan W, Faisal SM, Divers T, et al. Experimental *Leptospira interrogans* serovar Kennewicki infection of horses. *J Vet Intern Med* 2010;24:912–917.

21. Data on file, Study Report No. B951R-US-13-043, Zoetis Inc.

22. Data on file, Study Report No. B951R-US-13-046, Zoetis Inc.

23. Troedsson M HT, McCue PM. Pregnancy loss. In: Smith BP, ed. *Large Animal Internal Medicine*. 2nd ed. St. Louis, Mo: Mosby;1996:252–257.

24. Frazer G. Disorders of the reproductive tract. In: Reed SM, Bayly WM, Sellon DC, eds. *Equine Internal Medicine*. 3rd ed. St. Louis, Mo: Saunders Elsevier;2010:1054–1057.


TAKE TIME
OBSERVE LABEL DIRECTIONS



zoetis™