# Prevention of Leptospiremia and Leptospiruria Following Vaccination With a DAPPv + 4-Way *Leptospira* Combination Vaccine

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## Background

Leptospirosis, characterized by high fever, anorexia, vomiting, abdominal pain, diarrhea, myalgia, polyuria/polydipsia, jaundice, epistaxis, hematuria, and/or reproductive failure, continues to cause considerable morbidity among infected canines. Direct transmission of *Leptospira* spp. occurs when dogs come into contact with infected urine or ingest infected tissue. After dogs become infected, the spirochetes circulate in the blood for several days,[1,3] where they cause extensive damage to the endothelium of small blood vessels (leptospiremia). After the leptospiremic phase, the spirochetes can further colonize various organs, including the kidneys, where dogs can become a carrier and potentially shed organisms in the urine for months (leptospiruria). *Leptospira interrogans* serovars Canicola and Icterohaemorrhagiae are traditional causative agents of canine leptospirosis, and while the use of bacterins have decreased the prevalence of the disease, significant morbidity can still be attributed to infection with these serovars. In addition, leptospirosis caused by serovar Pomona and *L kirschneri* serovar Grippotyphosa[2,4,6] are becoming more prevalent, which has spurred development of bacterins that also provide protection against these serovars.

## Aim of the Work

In this study, we combined inactivated *L interrogans* serovars Canicola, Pomona, and Icterohaemorrhagiae and *L kirschneri* serovar Grippotyphosa with Nobivac® Canine 1-DAPPv (Animal Health at Merck & Co., Inc., Kenilworth, NJ USA), a commercially available vaccine that contains modified live canine distemper virus, adenovirus, parainfluenza virus, and parvovirus. We then vaccinated dogs with the combination product and evaluated the ability of the vaccination to prevent leptospiremia and leptospiruria following challenge with viable organisms of each serovar.

## Methods

### Preparation of the bacterin

*L interrogans* serovars Canicola, Pomona, and Icterohaemorrhagiae and *L kirschneri* serovar Grippotyphosa isolates were cultured in Ellinghausen, McCullough, Johnson, Harris (EMJH) medium,[5] heat-killed, and concentrated. All 4 serovars were then combined in a 1-mL volume of balanced salt solution that contained gentamicin, amphotericin B, and adjuvant. A 1-mL volume of the bacterin was then used to reconstitute 1 dose of Nobivac® Canine-1 DAPPv.

### Vaccination

Four separate studies were conducted. In each study, 24 7- to 8-week-old purpose-bred beagles (Ridglan Farms, Mount Horeb, WI) were vaccinated with the DAPPv + $L_4$ vaccine, and 12 dogs were vaccinated with placebo. The dogs were housed communally during the prevaccination, vaccination, and postvaccination phases and then segregated into individual cages for the challenge phase of the study. Food and water were provided ad libitum, and the experimental protocols were reviewed and approved by the Merck & Co., Inc., Kenilworth, NJ USA, Animal Care and Use Committee.

### Challenge with *Leptospira*

Dogs were challenged 2–3 weeks after the booster with a heterologous strain of either *L interrogans* serovars Pomona, Canicola, or Icterohaemorrhagiae or *L kirschneri* serovar Grippotyphosa. Prior to challenge, dogs were sedated, and then the challenge material was administered by dropping a 100-µL volume of EMJH medium that contained approximately $10^8$ spirochetes into each eye and intraperitoneal injection of approximately $10^9$ spirochetes in 4.5 mL of EMJH medium.

### Collection of blood and urine

Blood samples were collected by venipuncture at 3, 5, 7, and 10 days postchallenge. Prior to collecting urine on days 9/10, 13/14, 16/17, 20/21, 23/24, 27/28, 29/31, and 35 postchallenge, dogs were sedated, and then urine samples were collected by cystocentesis or catheterization.

### Detection of spirochetes

Spirochetes were detected by inoculating urine or blood (1:10) into EMJH that also contained 1% rabbit serum. The cultures were then incubated at 29°C and examined after 2 weeks by dark-field microscopy. Cultures that remained negative were subcultured (10-fold dilution) into fresh EMJH medium + 1% rabbit serum, and the original and subculture were re-incubated and examined biweekly for an additional 6 weeks. Recovery of spirochetes from one or more cultures was considered confirmation of leptospiruria or leptospiremia.

## Results

Spirochetes were not recovered from the blood samples collected from the vaccine recipients (**Table 1** and **Figure 1**). In contrast, spirochetes were detected from each blood sample collected 3 days postchallenge from the placebo recipients (n=12; 100%) challenged with either serovar Canicola, Icterohaemorrhagiae, or Grippotyphosa, and 9 (75%) blood samples collected 3 days postchallenge from the dogs challenged with serovar Pomona. In addition, with the exception of dogs challenged with serovar Pomona, *Leptospira* organisms were often recovered from blood samples collected up to 10 days postchallenge.

Similarly, spirochetes were not recovered from the urine samples collected from the vaccine recipients (**Table 2** and **Figure 2**). In contrast, spirochetes were recovered from at least two urine samples collected from the placebo recipients challenged with serovar Canicola (n=11; 100%), Grippotyphosa (n=11; 100%), or Icterohaemorrhagiae (n=10; 80%). In addition, spirochetes were recovered from at least one urine sample collected from the placebo recipients challenged with serovar Pomona (n=12; 50%). Many infected dogs continued to shed organisms in the urine for up to 35 days postchallenge. The collective findings therefore confirmed that the combination vaccine prevented blood or urine infection by each of the *Leptospira* serovars.

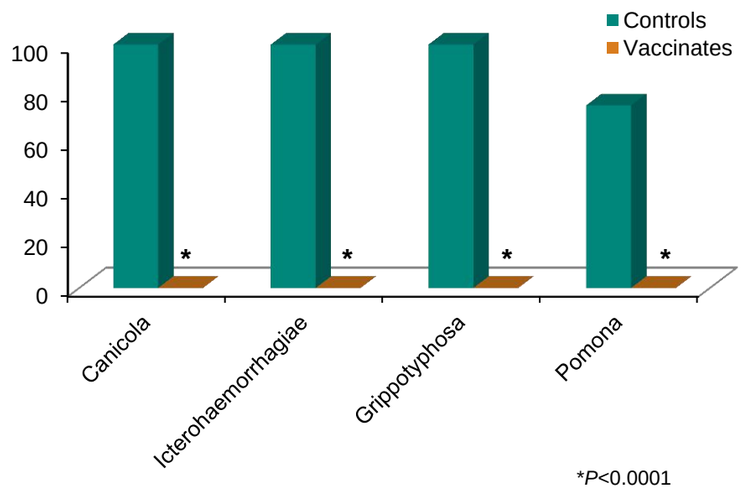
**Table 1. No. of dogs in which *Leptospira* was isolated from blood**

| Serovar | Treatment Group | Day Postchallenge | | | |
|---|---|---|---|---|---|
| | | 3 | 5 | 7 | 10 |
| **Canicola** | Vaccinates | 0/24 | 0/24 | 0/24 | 0/24 |
| | Controls | 12/12 | 9/11 | 6/11 | 3/11 |
| **Ictero** | Vaccinates | 0/24 | 0/24 | 0/24 | 0/24 |
| | Controls | 12/12 | 6/12 | 2/10 | 0/10 |
| **Grippotyphosa** | Vaccinates | 0/24 | 0/24 | 0/24 | 0/24 |
| | Controls | 12/12 | 8/12 | 4/11 | 0/11 |
| **Pomona** | Vaccinates | 0/24 | 0/24 | 0/24 | 0/24 |
| | Controls | 9/12 | 0/12 | 0/12 | 0/12 |

**Table 2. No. of dogs in which *Leptospira* was isolated from urine**

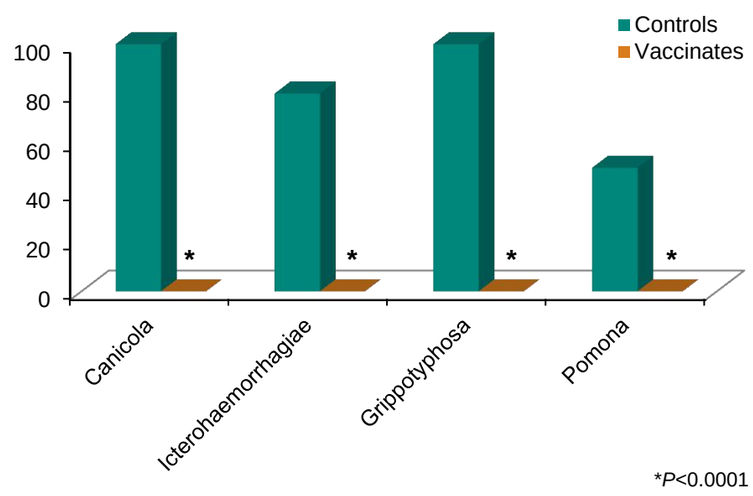
| Serovar | Treatment Group | Day Postchallenge | | | | | | | | Total No. of Dogs |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 9/10 | 13/14 | 16/17 | 20/21 | 23/24 | 27/28 | 29/31 | 35 | |
| **Canicola** | Vaccinates | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | **0/24** |
| | Controls | 9/11 | 11/11 | 9/11 | 8/11 | 10/11 | 9/11 | 8/11 | 8/11 | **11/11** |
| **Ictero** | Vaccinates | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | **0/24** |
| | Controls | 6/10 | 6/10 | 5/10 | 7/10 | 6/10 | 4/10 | 3/10 | 2/10 | **8/10** |
| **Grippotyphosa** | Vaccinates | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | **0/24** |
| | Controls | 2/11 | 5/11 | 6/11 | 8/11 | 7/11 | 8/11 | 7/11 | 1/11 | **11/11** |
| **Pomona** | Vaccinates | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | **0/24** |
| | Controls | 3/12 | 1/11 | 1/11 | 2/11 | 3/11 | 1/11 | 0/11 | 0/11 | **6/12** |

**Figure 1. Percentage of dogs that had *Leptospira* isolated from blood**




*P<0.0001

**Figure 2. Percentage of dogs that had *Leptospira* isolated from urine**




*P<0.0001

## Discussion/Conclusions

Leptospirosis continues to cause considerable morbidity among infected dogs. Moreover, while *L interrogans* serovars Canicola and Icterohaemorrhagiae are the widely recognized causative agents of canine leptospirosis, infections from serovar Pomona and *L kirschneri* serovar Grippotyphosa are becoming increasingly prevalent. Due to its zoonotic nature, the shedding of *Leptospira* organisms in the urine of infected dogs continues to be an important risk. The findings of this study confirmed that vaccination with Nobivac® Canine 1-DAPPv in combination with the $L_4$ bacterin effectively prevents leptospiremia and leptospiruria due to each of these serovars.

**References**

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