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, 2 When they cause extensive spirochetemia, they lead to disseminated disease (leptospirosis). After the leptospirosis 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 has 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 3-6 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® DAPPv + L2 (AH Animal Health at Merck & Co., Inc., Kenilworth, NJ USA), a commercially available vaccine that contains modified live canine distemper virus, adenovirus, parainfluenza & Co., Inc., Kenilworth, NJ USA). All rights reserved. 4.5 mL of EMJH medium, containing approximately 10^6 spirochetes in 4.5 mL of EMJH medium. Leptospira spp. are often isolated from blood samples collected up to 35 days postchallenge. The collective findings therefore support the vaccination to prevent leptospirosis and leptospiruria following challenge with viable organisms of each serovar.

Preparation of the bacterin

L interrogans serovars Canicola, Pomona, and Icterohaemorrhagiae and L. kirschneri serovar Grippotyphosa isolates were cultured in Eilenburghausen, McCullough, Johnson, Harris (EMJH) medium, 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 adventan. 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 + L2 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 Leptospirosis

Dogs were challenged 2-3 weeks after the booster with a heterologous strain of either L. interrogans serovars Canicola, Pomona, 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 10^6 spirochetes into each eye and intraperitoneal injection of approximately 10^8 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.

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 L2 bacterin effectively prevents leptospirosis and leptospiruria due to each of these serovars.