

One-Year Duration of Immunity in Dogs for *Leptospira Interrogans* Serovar Icterohaemorrhagiae After Vaccination

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KEY WORDS: leptospirosis vaccination, dog, duration of immunity, Canigen

ABSTRACT

Little information is available regarding the duration of immunity achieved by *Leptospira* vaccination. The aim of this study was to assess in dogs the one-year protection induced by a leptospirosis vaccine against a *Leptospira* (*L.*) *interrogans* serovar icterohaemorrhagiae challenge. Six 8-9 week old dogs were immunised twice at a 3-week interval with a bivalent vaccine composed of inactivated bacterins of *L. interrogans* serovar canicola and *L. interrogans* serovar icterohaemorrhagiae (CANIGEN® L, VIRBAC, France) and served as the vaccinated group. Six age-matched dogs were used as the control group. All dogs were challenged with *L. interrogans* serovar icterohaemorrhagiae 52 weeks after the primary vaccination. The control dogs developed mild clinical signs, leukocytosis, leptospiremia and became renal carriers as demonstrated by

positive results in urine culture and interstitial nephritis. In the vaccinated group, only one dog developed leptospiremia and shed leptospires in the urine. These results clearly demonstrated a significant one year protection by CANIGEN® L against leptospiremia, renal carrier state and urinary shedding after experimental infection with *L. interrogans* serovar icterohaemorrhagiae.

INTRODUCTION

Leptospirosis is a zoonotic disease of worldwide significance that affects many animal species and is caused by infection with antigenically distinct serovars of the species *Leptospira interrogans sensu lato*, of which at least 10 are of significant importance for dogs.¹ The serovars canicola and icterohaemorrhagiae are historically associated with clinical disease in dogs and thus are used as components of the commercial vaccines available from the 1970s. *Leptospira* (*L.*) *interrogans* serovar icterohaemorrhagiae has been shown to be the

most prevalent serovar in dogs and humans in many European countries due to the high infection pressure from rats which are the reservoir of this serovar.^{2,3} Given the ubiquitous nature of its maintenance host and the severity of the clinical signs, vaccination is always recommended to protect dogs against natural infection by *L. interrogans* serovar icterohaemorrhagiae.⁴ Two studies were published with contrasting results regarding the assessment of the long-term protection against challenge with *L. interrogans* serovar icterohaemorrhagiae after dog vaccination.^{5,6} This study investigated the one-year efficacy provided by the *L. interrogans* serovar icterohaemorrhagiae component of CANIGEN® L.

MATERIALS AND METHODS

Animals

Twelve conventional Beagle dogs of 8-9 weeks of age were randomly assigned to vaccinated and control groups (6 animals/group). None of the puppies had detectable antibodies to *L. interrogans* serovar icterohaemorrhagiae before immunisation. The puppies were maintained in two separate and isolated rooms. The puppies were fed once a day with a commercial diet and water was available *ad libitum*. The study was approved by the internal Ethics Committee and was conducted in compliance with the requirements of the European Convention for the protection of vertebrate animals for experimental purpose.

Vaccines

CANIGEN® L (VIRBAC S.A., Carros, France) is an inactivated and non-adjuvanted liquid vaccine containing whole-cell bacterins of *L. interrogans* serovars icterohaemorrhagiae and canicola and is mixed before use with CANIGEN® DHPPi (VIRBAC S.A., Carros, France). The latter is a lyophilised vaccine containing attenuated live canine distemper virus, canine adenovirus type 2, canine parvovirus and canine parainfluenza virus. The combination vaccine is commercially available as CANIGEN® DHPPi/L.

Study Design

Puppies received a primary vaccination of two doses (subcutaneously) of either CANIGEN® DHPPi/L (vaccinated group) or CANIGEN® DHPPi (control group) at a 3-week interval. Blood was sampled for serological examination prior to each vaccination and regularly throughout the 1-year vaccination period. Fifty two weeks after the second vaccine injection (day 0), each dog was challenged via the intraperitoneal route with approximately 3×10^9 organisms of *L. interrogans* serovar icterohaemorrhagiae strain I 109/90A.⁷ A clinical examination and body temperature recording were carried out daily for 4 weeks after challenge. The body weight was checked weekly. For leptospira isolation, blood samples were drawn seven times (days 0, 2, 3, 4, 5, 8, 11), urine samples were collected eight times (days 0, 3, 5, 8, 11, 14, 21, 28) and the right kidney was collected at the end of the study (day 28 post challenge). Blood was also sampled seven times for serological and haematological analyses (days 0, 4, 7, 11, 14, 21, 28). On the 28th day after infection, dogs were euthanised and necropsied. Tissue samples from kidney (left) and liver were collected for histological examination.

Serology

Sera were tested for antibodies against *L. interrogans* serovar icterohaemorrhagiae for the vaccinal and challenge periods. The antibodies were detected by means of the microscopic agglutination test (MAT) and the MAT titre is expressed as the reciprocal of the highest serum dilution that agglutinates 50% or more of the Leptospire relative to the control.⁸

Haematology

The white blood cell (WBC), red blood cell (RBC) and platelet counts and haematocrit were obtained by use of a Beckman Coulter AcT Diff hematology analyser (GMI, Ramsey, USA). For each haematological parameter, individual values were obtained from all the dogs sampled prior to the inoculation (day 0) and are expressed as a range about the mean including two standard

Table 1. Results of cultures after challenge of dogs with *L. interrogans* serovar icterohaemorrhagiae

| Group | Dog no. | Cultures ^a | | | | | | | | | | | | |
|------------------|---------|-----------------------|---|---|---|---|----|-------|---|---|----|----|----|----|
| | | Days after challenge | | | | | | | | | | | | |
| | | Blood | | | | | | Urine | | | | | | |
| | | 2 | 3 | 4 | 5 | 8 | 11 | 3 | 5 | 8 | 11 | 14 | 21 | 28 |
| Vaccinated Group | H3J025 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | H3J029 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | H3J030 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | H3J034 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | H3J035 | + | - | - | - | - | - | - | - | - | - | - | + | - |
| | H3J042 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Control Group | H3J020 | + | - | + | - | - | - | - | - | - | - | - | - | - |
| | H3J024 | + | - | - | - | - | - | - | - | + | + | + | + | - |
| | H3J031 | + | - | - | - | - | - | - | - | - | C | C | - | - |
| | H3J039 | + | - | - | - | - | - | - | - | + | - | + | + | - |
| | H3J054 | + | - | - | - | - | - | - | C | - | - | - | - | - |
| | H3J065 | - | - | - | - | - | - | - | - | + | + | + | + | + |

^a Cultures: (-) negative; (+) positive; (c) contaminated

deviations. These base lines were used to check that each individual value was normal during the challenge period.⁹

Blood, Urine, Kidney Cultures

For each dog, 0.5 ml of plasma sample was inoculated into a 25 cm² culture flask containing 9.5 ml of BSAT medium (in-house EMJH medium) plus 40 µl of 5-fluorouracil. The flask was incubated at 30°C. Cultures were then checked weekly for evidence of growth of leptospira by an increase in turbidity and by examination under dark-field microscopy between 2 and 6 weeks after inoculation (before being discarded as negative). The urine sample (0.5 ml urine) was put first into BSAT medium (4.5 ml) before being inoculated into a culture flask containing 5 ml of BSAT medium. Urine cultures were carried out as described above for blood cultures. At autopsy, the right kidney was taken and perfused with 20 ml of BSAT medium. The fluid harvested was put into a flask and cultured as described above for blood cultures.

Histological Examination

The left kidney and liver were collected post mortem and samples were fixed with formalin. Histological sections were stained with haematoxylin-eosin.

Statistical Analysis

Statistical analyses were performed using S-PLUS® 6.2 statistical software. Differences in haematology values between vaccinated and control groups were analysed using a two-sided student's t-test at each time point. The number of positive blood or urine cultures per dog were compared between vaccinated and control groups using a one-sided Wilcoxon rank sum test. The proportion of dogs with microscopic lesions was compared using a one-sided Fischer's exact test. P ≤ 0.05 is considered as significant.

RESULTS

After challenge with *L. interrogans* serovar icterohaemorrhagiae, all vaccinated dogs remained healthy. In the control group, clinical signs were mild in three dogs (fever of 39.6 °C and/or ocular mucosa congestion)

or absent. The control group also showed a slight decrease of platelets within the first week post infection (PI) and had a leukocytosis that was statistically different between groups 11 days ($P = 0.0441$) and 14 days ($P = 0.0452$) post infection. Post mortem examination revealed no gross lesions in any dog.

For all dogs, results of leptospira isolation in blood and urine were negative before challenge. Leptospiremia was observed within the first 3 days PI in five control animals and one vaccinated dog. Urinary shedding was recorded from the second week PI (day 8) in most control animals ($n=4$) and one vaccinated animal. Only one control dog had already detectable leptospira in urine on day 5 (Table 1). Leptospira infection could not be confirmed in any kidney samples. The number of positive cultures per dog is statistically different between the vaccinated group and the control group for blood ($P = 0.0163$) and urine ($P = 0.0368$).

The histological examination of the kidneys revealed tubules with basophilic epithelium and a mixed inflammatory infiltrate consisting of lymphocytes, plasmocytes and macrophages in four control animals. This interstitial nephritis was either moderate (2 dogs) or severe (2 dogs) and was observed in the renal cortex. It was also identified in the pyelic area in two dogs showing severe lesions. No significant lesions were observed in the livers of the control group. None of the vaccinated dogs showed significant histological lesions of kidney or liver. The proportion of the dogs with interstitial nephritis is statistically different between vaccinated and control groups ($P = 0.0303$).

After the primary vaccination course with CANIGEN® DHPPi/L, the mean antibody titre to *L. interrogans* serovar icterohaemorrhagiae peaked 2 weeks after the second injection and then subsequently declined to become very low within 15 weeks after vaccination. At the time of the challenge one year after the primary vaccination, one dog was still seropositive (MAT titre = 1/40). All control dogs remained seronegative during

the 1-year serological follow up. The challenge induced a strong increase of antibodies which was similar in both groups.

DISCUSSION

The aims of vaccination against leptospirosis are to protect dogs against clinical signs and also to prevent the renal carrier state.^{2,10,11}

After experimental (or natural) infection by leptospire, the clinical severity of the leptospirosis in dogs varies from a subclinical disease to severe disease with a fatal outcome. Mild and moderate illness are also observed.^{12,13} In fact, the majority of leptospiral infections in dogs are chronic or subclinical even though they are systemic.¹ Therefore a clinical leptospirosis is difficult to reproduce in dogs under experimental conditions especially when the number of tested animals is low. A few studies in puppies demonstrated a protection early after a primary vaccination against a lethal challenge of *L. interrogans* serovar icterohaemorrhagiae.^{6,7,12} In these studies, control puppies died after having shown severe clinical signs such as hypothermia, depression, anorexia, dehydration, icterus, abdominal pain, weight loss, vomiting and diarrhoea. These symptoms and death, as observed in *L. interrogans* serovar icterohaemorrhagiae-infected dogs, correlated with renal failure as demonstrated by a sharp increase of urea and creatinine.⁷

Regarding the long term immunity, two studies have already been conducted to demonstrate a one-year protection in dogs against *L. interrogans* serovar icterohaemorrhagiae with contrasting results.^{5,6} No evidence of clinical signs was seen in the first study,⁵ while severe leptospirosis with fatal outcome was observed in the second one.⁶ Unlike the puppies in our previous study,⁷ the adult control dogs did not develop severe disease in this study while the *L. interrogans* serovar icterohaemorrhagiae inoculum was similar (challenge strain, culture method of challenge strain, challenge dose).⁷ The lower susceptibility of adult animals to infection and/or the loss of factors related to virulence of the challenge strain after multiple passages in culture could explain the mild

symptoms and the mild clinico-pathological changes recorded after challenge.¹⁴

The prevention of the renal carrier state is the second aim of vaccination against leptospirosis. Indeed renal colonization often occurs, is responsible for large numbers of leptospire in the urine, is long-lasting and is rarely diagnosed. This renal colonization occurs in most infected animals because the organisms replicate and persist in renal tubular epithelial cells, even in the presence of serum neutralizing antibodies.¹

The largest numbers of leptospire in urine are related either to the early stage of leptospiremia (as soon as day 2 PI) or to the renal carriage from day 14 PI after experimental inoculation of *L interrogans* serovar icterohaemorrhagiae or *L interrogans* serovar canicola.¹¹ The recovered dogs can excrete leptospire in their urine intermittently for months after infection.¹ Finally, as most leptospiral infections in dogs are subclinical or chronic,¹ they are typically not diagnosed and treated. Therefore prevention of urine shedding by vaccination is essential due to the zoonotic risk. *L interrogans* serogroup icterohaemorrhagiae is of primary importance in this respect as it is the most prevalent serogroup in dogs in Europe.²⁻⁴

A few studies in vaccinated dogs have demonstrated a prevention of renal carriage and urinary shedding shortly after challenge with *L interrogans* serovar icterohaemorrhagiae.^{6,7,10,11,15} In these short-term studies, the negative results for culture isolation from urine and kidney samples are also associated with negative results for blood culture. One long term study has demonstrated a prevention of renal carriage and urinary shedding 14 months after challenge with *L interrogans* serovar icterohaemorrhagiae and a significant reduction of the urinary shedding with *L interrogans* serovar canicola.⁶ In both challenges, leptospire were isolated from blood. In our study, we have also observed a significant reduction of urine shedding as only one vaccinated dog (out of 6) was tested positive for urine isolation with *L interrogans* serovar icterohaemorrhagiae.

This dog was also positive for blood culture. Such results, in combination with the complete absence of clinical disease in vaccinated dogs, support the rationale for a 1-year revaccination interval with CANIGEN® L.

After being given a primary vaccination course with CANIGEN® L, dogs developed low ($\leq 1/80$) and short lived (1 to 4 months) MAT titres to *L interrogans* serovar icterohaemorrhagiae so that most of them were seronegative at the time of challenge performed one year later. Therefore these antibody titres do not correlate with protection as the seronegative vaccinated animals were still protected against a *L interrogans* serovar icterohaemorrhagiae challenge. All these results are in accordance with previous short- and long-term studies using distinct serovars of *Leptospira* spp.^{5-7, 16-18}

CONCLUSION

A primary vaccination course comprising two doses of CANIGEN® L given 3 weeks apart provides a one year duration of protection from leptospiremia, the renal carrier state and urinary shedding against challenge with *L interrogans* serovar icterohaemorrhagiae.

ACKNOWLEDGMENTS

The authors thank David McGahie for his critical reading of the manuscript.

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