

Duration of immunity and clinical protection against canine leptospirosis with a multivalent vaccine

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SUMMARY

In order to investigate the duration of immunity against canine leptospirosis conferred by a multivalent vaccine, Canigen® DHPPi/L, Beagles received two subcutaneous vaccine injections three weeks apart. Resulting serological response, using MicroAgglutination Test (M.A.T.), was followed during one year. One year after vaccination, vaccinated dogs were challenged with one of the two main pathogenic strains of leptospira in dogs, *L. icterohaemorrhagiae* or *L. canicola*. Clinical, serological, haematological and biochemical survey was then performed. Following vaccination, serological response appeared to be weak and transient. Nevertheless, unlike unvaccinated control dogs, vaccinated dogs resisted to challenge one year after use of standard vaccination protocol without any booster.

Keywords : leptospirosis - dogs - vaccination - protection – immunity.

Introduction

Leptospirosis is a worldwide zoonosis affecting many animal species and caused by *Leptospira interrogans* [2,7,10]. The serological classification of the members of this species distinguishes 23 serogroups divided into more than 200 serovars [2]. Dog is particularly sensitive to this disease and two serovars were determined to be predominant for canine leptospirosis : *Leptospira interrogans canicola* and *leptospira interrogans icterohaemorrhagiae* [2,3,10]. The two main forms of the canine leptospirosis are haemorrhagic gastro-enteritis principally due to *L. canicola* and haemorrhagic icterus usually due to *L. icterohaemorrhagiae* [2]. This etiologic predominance led to the elaboration of vaccines containing inactivated bacterins of these two serovars [2,3,10].

Few data relative to the immunity duration conferred by vaccination against leptospirosis in dogs are available. Many authors advised revaccination of dogs every six months and even more frequently in endemic areas [3,7,10]. This implicitly supposes that vaccine efficacy just allows protection during short period. That's why this study was planned in order to evaluate possibility to protect dogs against leptospirosis during effective period of one year using a standard protocol of primovaccination with a multivalent vaccine (Canigen® DHPPi/L, Virbac). Experiment was performed in Beagles via two following subcutaneous administrations of the vaccine 3 or 4 weeks apart. Immunity conferred by the vaccine was assessed by measurement of humoral response in vaccinated animals, using microagglutination test during one year. Clinical protection was assessed by resistance to challenge infection, using two virulent

strains of leptospira, *L. icterohaemorrhagiae* or *L. canicola*, one year after vaccination.

Materials and Methods

A) ANIMALS

Fourty-five 8-to-9-week-old SPF Beagle puppies, supplied by Liberty Research, U.S.A and housed at Chrysalis, FRANCE, were used for this experiment. They were divided into two groups, housed separately: a group of 35 dogs, which were vaccinated and a group of 10 dogs kept as controls. At the beginning of the trial, all dogs were tested and were serologically negative.

B) EXPERIMENTAL DESIGN

35 dogs were vaccinated by two injections of 1,0 ml of Canigen® DHPPI/L by the subcutaneous route 3 to 4 weeks apart. 10 unvaccinated dogs were kept as controls. Serological follow-up was performed weekly from the first vaccine injection to the fourth week after the second injection in order to evaluate the serological peak and then every month until month 11 after first vaccine injection.

Twelve months after the second vaccine injection, 20 dogs were challenged : 5 vaccinated and 5 control dogs were inoculated with the *icterohaemorrhagiae* strain and 5 vaccinated and 5 control dogs received *canicola* strain. Animals were kept under clinical control during 24 days. Blood samples were taken from D0, day of the challenge, to D21 every two, three or four days for serological investigation. At the same dates, blood samples were also obtained in order to evaluate the evolution of the haematological and biochemical parameters (ALT,

ALP). A measurement of the uremia and the creatinemia was also carried out at D0, D7, D14 and D21. At D24, puppies were euthanased. The kidneys of each dog were sampled for histological analysis and leptospira detection.

C) VACCINES

The commercial vaccine Canigen® DHPPi/L that has been used is composed of two vaccines to be combined prior to injection in dogs, i.e.:

- Canigen® DHPPi which is a freeze-dried multivalent vaccine (batch N° MP 28) containing following valences: distemper, contagious canine hepatitis, canine parvovirus and parainfluenza. All these valences consist in attenuated viruses produced on cell culture.

- Canigen® L that is a liquid vaccine (batch N° M179) containing bacterial valences of two canine leptospira serovars: *L. canicola* and *L. icterohaemorrhagiae*. They have been cultivated on synthetical medium and inactivated by sodium mercuriothiolate.

D) CHALLENGE INOCULUM

Two strains of *Leptospira interrogans* were used for challenge infections. They were originated from the Infectious Pathology Unit of the National Veterinary School of Nantes (ENVN), France.

- *icterohaemorrhagiae* serovar, *Icterohaemorrhagiae* serogroup, isolated in 1989 from a dog died of acute leptospirosis and determined by the Pasteur Institute, Paris.

- *canicola* serovar, *Canicola* serogroup provided by Dr. Mailloux of the Pasteur Institute, Paris.

Preparation of the inoculate was performed at the Infectious Pathology Unit. The two suspensions containing the serovar *icterohaemorrhagiae* and the serovar *canicola* had a titer of 120 units measured by turbidimeter, which amounts to concentration of $2 \cdot 10^9$ bacteria/ml.

Dogs were anaesthetized by intramuscular injection with Zoletil 100® (Virbac) at a posology of 0,1ml/kg; then, inoculated with 5 ml of the bacterial suspension in the inguinal canal.

E) CLINICAL FOLLOW-UP

Dogs were carefully monitored every day and were particularly observed for the presence of typical signs of leptospirosis. Clinical scores were attributed to dogs according to intensity of these clinical signs as described in table I.

F) SEROLOGY

Blood was drawn from the jugular vein of the dogs in order to obtain at least 2×2 ml of serum. The anti-leptospira antibody analyses were performed at the ENVN using the reference test, i.e. M.A.T. carried out according to internal procedures of the Infectious Pathology Laboratory of the ENVN [2,6]. The sera of all dogs were tested for antibodies against serovars *icterohaemorrhagiae* (strain 19) and *canicola* (can). After challenge, homologous response was essentially considered.

Antibody titre is expressed as the reciprocal of the highest dilution of serum at which at least 50% of the leptospira were agglutinated. Limit of quantification was determined as 1/40, limit under which no difference between 0 and 1/40 can be distinguished.

G) BIOCHEMISTRY AND HAEMATOLOGY

Evolution of biochemical parameters, including alanine transferase (ALT), alkaline phosphatase (ALP), uremia and creatinemia, was measured on blood samples after challenge using standard techniques. Haematological analyses were also performed using automated analyzer.

H) NECROPSY EVALUATION

A complete necropsy was performed on all dogs. Kidneys were sampled to microscopic evaluation of histologic sections and for search for of leptospira.

I) PRESENTATION OF RESULTS AND STATISTICAL ANALYSIS

In order to simplify the presentation, all antibody titres are expressed as the arithmetic mean of the log₁₀ of the reciprocal titres and represented as a linear function of time in weeks.

The average clinical scores were compared between each group using monolateral Mann Whitney test.

Results

A. SEROLOGICAL RESPONSES

Serological response directed against serovar *icterohaemorrhagiae* was variable. The peak generally appeared at week 6, i.e. 2 weeks after the second vaccine injection (fig. 1). Four dogs did not respond and two others were just at the limit of quantification. At week 16, i.e. three months after vaccination, 28 from 35 dogs had a serological response negative or just at the limit of quantification. From week 39, all samples were seronegative. After *icterohaemorrhagiae* challenge, a quick and high antibody response directed against this serovar was shown, reaching its maximum value (mean titre 1/954) at D10 (fig. 2). Control dogs displayed a slower seroconversion and a maximum response only the last week of challenge.

All dogs displayed a serological response directed against *canicola* serovar. The peak, which appeared at week 6 after vaccination, was higher than with the *icterohaemorrhagiae* serovar and it lasted longer. At week 16, 32 from 35 animals had a serological response negative or just at the limit of quantification. After *canicola* challenge, all dogs either vaccinated, either control, displayed a rapid and important rise of homologous antibodies titre.

B) CLINICAL FOLLOW-UP

In *L. icterohaemorrhagiae* challenge, all control dogs displayed variable clinical signs. A general loss of energy was noticed in all dogs with abdominal pain, yellowish coloring of the mucosa and dark yellow urine. Two dogs displayed more serious symptoms with development of an icterus and petechia.

Among vaccinated dogs, two dogs displayed sporadic clinical signs, including hyperthermia, abdominal pain and hypertrophy of the liver evaluated by abdominal palpation. These signs decreased spontaneously and did not affect the general health state of the dogs. The difference in clinical scores between vaccinated and control dogs which were infected with *L. icterohaemorrhagiae* was clearly significant ($p = 0,01$) (fig. 4).

In *L. canicola* challenge control animals displayed variable clinical signs. One control dog challenged with *L. canicola* displayed serious symptoms, as an icterus with a yellowish coloration of the mucosa, anorexia, depression, persistence of the skin fold, hypersalivation and two epileptic fits. It was euthanized at D6. The four other control dogs of this challenge constantly showed a yellowish coloration of the mucosa, a liver hypertrophy and abdominal pains but these signs appeared at different times. Vaccinated dogs displayed a weak icterus and three animals had a liver hypertrophy noticed at palpation. One dog showed a slight hyperthermia associated with a loss of energy and then abdominal pain and dehydration. All these signs decreased spontaneously and the general health state of these dogs was considered as satisfactory. The difference in clinical scores between vaccinated and control dogs which were inoculated by *L. canicola* challenge was less important than with *L. icterohaemorrhagiae* challenge but remained significant ($p=0.037$) (fig.5).

C) HAEMATOLOGICAL AND BIOCHEMICAL FOLLOW-UP

Three control dogs displayed leucopenia followed by leucocytosis. A decrease in the concentration of platelets was observed in control groups and in vaccinated group being infected by *L.icterohaemorrhagie*; it nevertheless remains weak and transient. A slight increase in ALP was noticed in most of the dogs from each group. Others biochemical or haematological parameters remained on the whole within the physiological norms.

D) NECROPSIC EXAMINATION

The histological analysis of the kidneys sampled among vaccinated and control dogs euthanized on D24 did not reveal any significant lesion consistent with the evolution of an acute or subacute leptospirosis. In the opposite, impairments typical of leptospirosis were detected in the control dog challenged by *L. canicola* and euthanized at D6 including severe intestinal nephritis lesions, necrotizing glomerulitis and vasculitis.

No isolation of leptospira was possible from kidneys sampled at the end of the trial but leptospira were isolated from the kidney of the dog euthanized at D6.

Discussion

The serological response after vaccination appeared to be transient, roughly 4 to 5 months, and weak. Antibody titre against the three considered serovars declined below the detection level within four months after second vaccinal injection. The highest antibody response was obtained for *canicola* serovar. This may be representative of a greater antigenic content of the *canicola* component in the vaccine. M.A.T. allows detection of agglutinating antibodies, essentially represented by IgM, which are the first antibodies produced after immunization. They are often reported to decrease rapidly after vaccination [4,7]. IgM antibody titre may not be related to immunity protection level.

Following challenge, non vaccinated control dogs from both groups, i.e. inoculated by *L. canicola* or *L. icterohaemorrhagiae*, displayed clinical signs of leptospirosis with delayed apparition and variable intensities. One animal was sacrificed six days after inoculation of *L. canicola* serovar *canicola* as it showed an acute renal insufficiency with intoxication by urea. On a general point of view, vaccinated dogs showed some transitory signs that, when observed, decreased spontaneously. The difference in clinical scores between vaccinated and control dogs is significant whatever the considered challenge. Both challenges did not display the same virulence; a mean clinical score of 2.8 was obtained for dogs challenged with *L. icterohaemorrhagiae*, whereas this mean score was of 7 among dogs challenged with *L. canicola*. It is quite difficult to reproduce a clear clinical expression of leptospirosis under experimental conditions by inoculating dogs

with virulent strains of leptospira [9]. Results obtained in this study, observation of clinical signs typical of leptospirosis in control groups and development of acute leptospirosis in one control dog challenged by *L. canicola*, were therefore considered as satisfactory.

Finally, in both cases, the vaccinated dogs displayed, after challenge, a rapid increase of homologous antibody titres that can be explained by existence of memory immunological response. This anamnestic response was less pronounced for *canicola* serovar.

Some authors have taken an interest in the protective IgG antibody production, using ELISA test, after vaccination in dogs. Results demonstrated the quick decrease of IgG level after two vaccinal injections three weeks apart and therefore the need for a third or even a fourth vaccination in the initial series, with annual revaccination [5]. In this experiment, leptospira challenge was performed one year after only two vaccinal injections and dogs were found to be protected against clinical leptospirosis without any booster vaccination. This may reflect role of cell-mediated immunity in protection against leptospirosis and secondary role of humoral response.

Efficacy of vaccines against canine leptospirosis must be tested, according to European pharmacopoeia norms, in hamsters, which are a particularly sensitive species to the disease [5,8]. Hamsters are vaccinated with 1/10 of the canine vaccinal dose and challenged by virulent strains three or four weeks later. Survival rate is investigated. These tests present therefore limits as they are performed on species different from target species using a weakly diluted vaccine.

Some studies demonstrated correlation between protection of dogs and hamsters during a short period: challenges were performed three or four weeks after vaccination for both species. Long term immunity conferred by vaccination in hamsters was assessed by resistance to challenge infection 18 months following two vaccinal injections [7]. To our knowledge, this is the first reported similar study performed on dogs which resisted to challenge infection one year after two injections of primovaccination.

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Table I – clinical scores scale attributed to dogs according to observed clinical signs

Clinical signs	Intensity	Scores
fever	<37,5 or >39,5	1
depression	slight	1
	moderate	2
	significant	3
dehydration	slight	1
	moderate	2
	significant	3
aspect of mucosa	red	1
	yellowish	2
	icterus	3
liver hypertrophy		1
abdominal pain	slight	1
	moderate	2
	significant	3
vomiting		1
epileptic fit		5
death		10

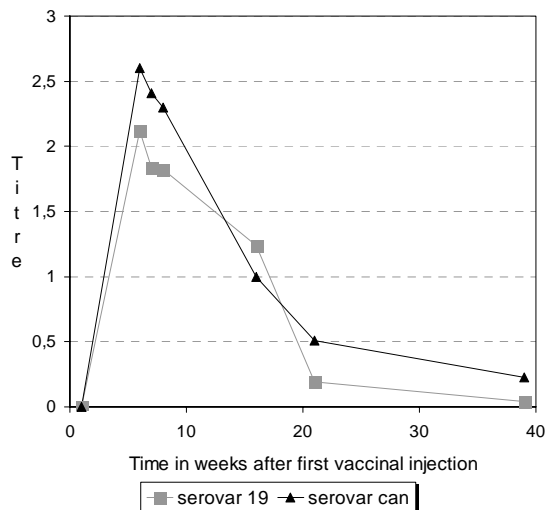


Figure 1 – Mean of serological values for *icterohaemorrhagiae* and *canicola* after vaccination

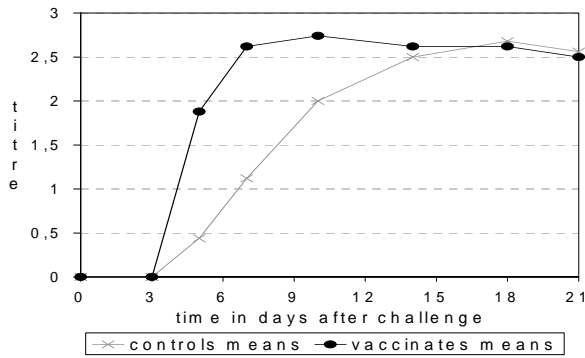


Figure 2 – serologic response against serovar *icterhaemorrhagiae* during the *icterohaemorrhagiae* challenge

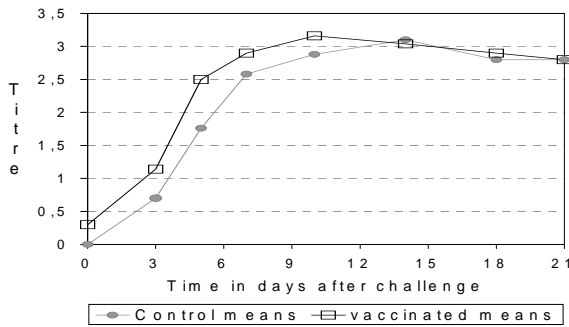


Figure 3 – serologic response against serovar *canicola* during the *canicola* challenge

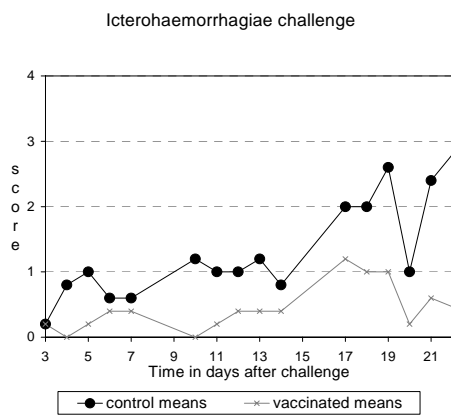


Figure 4 – Daily mean clinical scores of dogs challenged with *icterohaemorrhagiae* strain

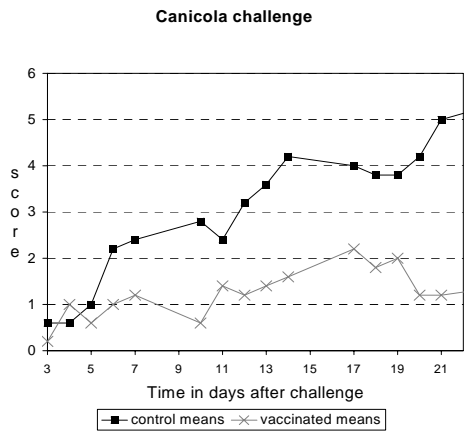


Figure 5 – daily mean clinical scores of dogs challenged with *canicola* strain