Prevention of a severe disease by a Leptospira vaccination with a multivalent vaccine

P. SCHREIBER*, V. MARTIN, W. NAJBAR, A. SANQUER, S. GUEGUEN and B. LEBREUX

SUMMARY

Little information is available regarding the protection achieved by vaccination of dogs against a high virulent experimental inoculation of Leptospira although leptospirosis remains of clinical importance. The aim of this study was to assess in dog the protection induced by a commercial leptospirosis vaccine after a Leptospira interrogans serovar canicola (L. canicola) challenge. Twelve 8-9 week old Specific Pathogen Free (SPF) dogs were used for this study. Six animals were immunised twice at a 3-week interval with a bivalent vaccine composed of inactivated bacterins of L. canicola and L. icterohaemorrhagiae (vaccinated group). The six remaining dogs were left unvaccinated (control group). All dogs were challenged via the intraperitoneal route with L. canicola 5 weeks after the second vaccine injection and checked for 4 weeks. Five control dogs died within the 8 days post inoculation (PI). These five animals showed a similar clinical picture (fever, hyperthermia, depression, vomiting, anorexia, abdominal pain, icterus, diarrhoea, dehydration, haematuria, weight loss) and also similar gross lesions at autopsy (icterus, petechiae, haemorrhage, hypertrophy of kidneys). A leptospirosisemia was observed between day 2 and day 4 PI. The surviving control dog showed a mild disease. In opposition, all vaccinated dogs showed neither disease nor infection. Therefore, this leptospirosis vaccine is shown to achieve complete prevention of leptospirosisemia, death, clinical signs, gross and microscopic lesions after a virulent challenge with L. canicola.

Keywords : canine leptospirosis - vaccination - challenge - protection.

Introduction

Leptospirosis is a worldwide zoonosis caused by Leptospira interrogans which include all pathogenic strains of the Genus Leptospira. The serological classification of the members of Leptospira interrogans distinguishes more than 200 serovars which have traditionally been grouped into serogroups according to their antigenic relation [16]. After natural infection with Leptospira, the spectrum of infection in the dog ranges from acute to subacute or chronic [18]. These clinical forms are influenced by virulence factors, the immune status of the dog and the host adaptation (i.e. primary reservoir host or incidental host) [11]. The disease in primary reservoir hosts tends to be more chronic or asymptomatic with weak antibody responses. In contrast, the disease in incidental hosts tends to be acute and severe with marked antibody responses [18]. The common clinical signs expected in dogs include hypothermia, pyrexia, depression, anorexia, vomiting, abdominal pain, icterus, congestion of ocular mucosa, diarrhoea [13, 7, 1]. Historically, the serovars associated with clinical disease in the dog included L. canicola and L. icterohaemorrhagiae [10, 7, 18]. Therefore vaccines currently used in dogs in most countries contain inactivated whole Leptospira belonging to serogroups Canicola and Icterohaemorrhagiae [16, 18].

Recent clinical observations and serological surveys in Europe and North America demonstrated that new emergent serovars particularly sejroe, grippotyphosa and bratislava were associated with leptospiroal infection in dogs [20, 21, 19, 8]. However, the decrease in the prevalence of L. canicola and to a lesser extent L. icterohaemorrhagiae in dogs can be explained by the widespread use of vaccines containing these serovars in dogs [11, 5, 3, 1, 19]. The removal of this vaccination could increase the infection pressure of these serovars [2, 15]. Moreover, no data are available regarding the prevalence of chronic infections by these serovars in dogs as the microscopic agglutination test (used for the serological surveys) demonstrates mainly the recent infections [15]. Therefore, the dog vaccination with both serovars L.

RéSUMÉ

Prévention d’une symptomatologie sévère par la vaccination contre la leptospirose à l’aide d’un vaccin multivalent. Par P. SCHREIBER, V. MARTIN, W. NAJBAR, A. SANQUER, S. GUEGUEN et B. LEBREUX.

Peu d’information sont disponibles concernant la protection conférée par la vaccination des chiens vis-à-vis d’une inoculation d’épreuve à l’aide de leptospires alors que la leptospirose est toujours importante sur le plan clinique. Le but de cette étude fut d’évaluer chez le chien la protection induite par un vaccin commercial après infection expérimentale avec le sérovar canicola. Douze chiens SPF, âgés de 8 à 9 semaines, ont été utilisés pour cette étude. Six chiens ont été immunisés deux fois à 3 semaines d’intervalle par un vaccin comprenant les sérovars canicola et icterohaemorrhagiae (groupe vacciné). Les six autres chiens n’ont pas été vaccinés (groupe contrôle). Les chiens ont été ensuite inoculés à l’aide du sérovar canicola 5 semaines après la seconde injection vaccinale et ont été ensuite suivis pendant 4 semaines. Cinq chiens non vaccinés sont morts dans les 8 jours suivant le challenge. Ces chiens ont tous présenté un tableau clinique semblable (fièvre, hyperthermie, prostration, vomissement, anorexie, douleur abdominale, ictere, diarrhée, déshydratation, hématurie, perte de poids) et des lésions macroscopiques similaires (ictère, pétéchies, hémorragies, hypertrophie rénale). Une leptospirose a aussi été observée entre les deuxième et quatrième jours après le challenge. Un chien a survécu et a présenté une symptomatologie légère. Par contre tous les animaux vaccinés n’ont présenté ni signes cliniques ni infection. En conclusion, ce vaccin prévient la leptospirose, la mort, les symptômes et les lésions macroscopiques après challenge avec le sérovar canicola.

Mots-clés : leptospirose canine - vaccination - inoculation d’épreuve - protection.
canicola and *L. icterohaemorrhagiae* should be always considered as relevant. The aim of this study was to investigate in dog the protection provided by a commercial vaccine containing *L. canicola* and *L. icterohaemorrhagiae* components against a severe challenge with *L. canicola*.

**Material and methods**

**ANIMALS**

Twelve SPF Beagle puppies (6 males and 6 females) were obtained from a commercial supplier (Harlan Sprague Dawley, USA). The dogs were randomly assigned to 2 groups (vaccinated and unvaccinated) according to the number (n=6) and the sex ratio (1/1). They were housed in a P3-level animal facility and were 8-9 weeks old at the first vaccine injection day. All procedures were approved by internal Ethics Committee and were performed in compliance with the requirements of the European Convention for the protection of vertebrate animals used for experimental purpose.

**VACCINES**

CANIGEN® L (VIRBAC S.A., Carros, France) is a non-adjuvanted liquid vaccine containing a suspension of inactivated whole organisms of *L. canicola* and *L. icterohaemorrhagiae*. The vaccine is mixed before use with CANIGEN® DHPPi, a lyophilised vaccine containing live canine distemper virus, canine adenovirus type 2, canine parvovirus and canine parainfluenza virus. The combination vaccine is denoted CANIGEN® DHPPi/L.

**CHALLENGE STRAIN**

The ENVN strain, identified as belonging to *Leptospira interrogans* serogroup Canicola serovar canicola (“Centre National de Référence des Leptospires”, Institut Pasteur, Paris), was used for the challenge. A bacterin suspension with a titre of 1.17 x 10⁹ cells/mL was prepared in order to obtain an inoculum of 6 x 10⁹ organisms per dog.

**EXPERIMENTAL DESIGN**

Six dogs were immunised twice with a dose of CANIGEN® DHPPi/L at a 3-week interval (vaccinated group). Six dogs were left unvaccinated and were kept as control animals (control group) (Table I). Five weeks after the second vaccine administration, all animals were challenged with a dose of 5 mL of virulent strain via the intraperitoneal route (day 0) and were sacrificed 4 weeks PI (day 28). A veterinary clinical examination, including body temperature, was carried out once daily for 4 weeks. The body weight was recorded once a week after challenge (days 0, 7, 14, 21, 28). Blood samples were taken weekly PI in order to perform serology, haematology and biochemistry. In order to carry out *leptospirosis* isolation, blood was sampled six times PI (days 0, 2, 3, 4, 7, 11), urine samples were collected weekly by cystocentesis (days 0, 7, 14, 21, 28) and the right kidney was taken at the end of the study (day 28). A *post mortem* examination was performed and tissue samples were collected from kidney (left) and liver for histological examination. In case of early death (animal found dead or euthanised for ethical reasons), the autopsy, the collection of tissue samples and the histological examination were also undertaken.

**SEROLOGY**

Sera were tested by microscopic agglutination (MA) for antibodies against *L. canicola*. The MA titre is expressed as the reciprocal of the highest serum dilution that agglutinates 50% or more of the *Leptospira* relative to the control [16].

**HAEMATOLOGY**

The white blood cell (WBC), red blood cell (RBC) and platelet counts and haematocrit were obtained by use of Coulter Act Diff haematology analyser (Beckman Coulter). 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 deviations. These baseline values were used in order to check that each individual value was normal during the PI period [22].

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Disease severity</th>
<th>Clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>20916</td>
<td>severe, death on day 6</td>
<td>pyrexia, hypothermia, depression, anorexia, haematuria, vomiting, icterus, weight loss, abdominal pain, diarrhoea, dehydration</td>
</tr>
<tr>
<td>20925</td>
<td>severe, death on day 8</td>
<td>pyrexia, hypothermia, depression, anorexia, haematuria, vomiting, weight loss, melena, nasal discharge, abdominal pain, dehydration</td>
</tr>
<tr>
<td>20383</td>
<td>severe, death on day 4</td>
<td>pyrexia, hypothermia, depression, anorexia, icterus, abdominal pain, dehydration</td>
</tr>
<tr>
<td>20912</td>
<td>severe, death on day 6</td>
<td>pyrexia, hypothermia, depression, anorexia, haematuria, icterus, weight loss, abdominal pain, dehydration</td>
</tr>
<tr>
<td>20342</td>
<td>severe, death on day 5</td>
<td>pyrexia, hypothermia, depression, anorexia, haematuria, vomiting, icterus, abdominal pain, diarrhoea, dehydration</td>
</tr>
<tr>
<td>20913</td>
<td>mild, recovery</td>
<td>weight loss (day 14)</td>
</tr>
</tbody>
</table>

Table I. — Clinical signs in control group after challenge with *Leptospira interrogans* serovar canicola.

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*SCHREIBER (P.) AND COLLABORATORS*
BIOCHEMISTRY

Urea, total bilirubin, creatinine, alkaline phosphatase (AP), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were determined using OLYMPUS AU640 chemistry analyser. Like haematology, biochemical results were assessed according to baseline values.

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 plus 40 µl of 5-fluorouracil. The flask was incubated at 30°C. Cultures were checked at least 3 times for evidence of growth of *Leptospira* by an increase in turbidity and by examination under dark-field microscopy between 2 to 6 weeks (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 analysis were performed using NCSS® 2000 statistical software. Student’s *t* test was used for the assessment of the quantitative parameters (bodyweight, rectal temperature) on the challenge day. The time courses of these quantitative parameters were analysed using ANOVA 2 test on repeated measures. Analysis of survival probability of dogs after challenge was made using the Kaplan-Meier method. The Log-rank test was used to compare the survival curves of vaccinated and control groups. The number of positive blood or urine cultures per dog was compared between vaccinated and control groups using a Mann-Whitney test. The proportion of dogs with positive kidney culture per group and the proportion of dogs with gross or microscopic lesions was made using Fischer’s exact test. A *P*-value < 0.05 was considered significant.

Results

CLINICAL EXAMINATION

All vaccinated animals remained healthy throughout the 4-week study period. Five (out of six) unvaccinated dogs (nos. 20916, 20925, 20383, 20912, 20342) were euthanised or were found dead between days 4 and 8 PI and showed a similar clinical picture. After an early pyrexia (day 2 and/or day 3), these dogs showed severe clinical signs such as hypothe-

SEROLOGY

All dogs were free of specific antibodies against *L. canicola* before vaccination (day -55). Two weeks after the second vaccine injection (day -20), the six animals showed high individual antibody titres which either persisted or slightly decreased one week later. In both vaccinated and control groups, the experimental inoculation induced a strong antibody increase (on PI day 7 or 14) followed by an antibody decrease up to the end of the study. However, the serological response was higher in the control group in comparison with the vaccinated group (Figure 2).

HAEMATOLOGY

In control group, significant changes in haematological parameters i.e. values outside the baseline values were detected in 2 dogs. Dog no. 20925 showed on PI day 7 a leukocytosis (24.200 cells/mm³) and a decrease in RBC (4.56 10⁶ cells/mm³), haematocrit (34.1 %) and platelets (115.000 cells/mm³). A decrease in erythrocytes (5.01 10⁶ cells/mm³) on day 7 and a haematocrit decrease on days 7, 14 and 21 (34.7, 37.7, 37.1 %) was seen in the surviving dog no. 20913 (Table II). None of the vaccinated dogs had significant changes.

BIOCHEMISTRY

In unvaccinated group, values higher than the baseline values were seen on PI day 7 for urea (0.83 g/L) and in dog no. 20925 for urea (6.90 g/L), for creatinine (95 mg/L) and for total bilirubin (3.2 mg/L) (Table II). No abnormal results were recorded in vaccinated group.
BLOOD, URINE, KIDNEY CULTURES

In all control animals, the challenge strain was re-isolated in blood from day 2 PI during 2 (nos. 20342, 20913) or 3 consecutive days (nos. 20916, 20925, 20383, 20912) while all vaccinated dogs remained negative for blood isolation. The number of positive blood cultures per dog is statistically different between vaccinated group and control group ($P = 0.0022$). The surviving control dog no. 20913 and the vaccinated animals showed negative results for the presence of challenge strain in urine and kidney samples (Table II).

POST MORTEM EXAMINATION

In control group, two dogs were found dead on day 6 and three were euthanised on day 6 or day 8 and necropsied

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Haematological changes</th>
<th>Biochemical changes</th>
<th>Gross lesions</th>
<th>Histological lesions</th>
<th>Leptospira isolation (blood, urine, kidney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20916</td>
<td>ND</td>
<td>ND</td>
<td>kidney hypertrophy, liver discoloration, widespread icterus</td>
<td>haemorrhagic and necrotic pan-nephritis, liver autolysis</td>
<td>blood (days 2,3,4)</td>
</tr>
<tr>
<td>20925</td>
<td>RBC decrease, haematocrit decrease, leucocytosis, platelet drop (day 7)</td>
<td>urea increase, creatinine increase, T. bilirubin increase, (day 7)</td>
<td>kidney hypertrophy, liver discoloration, widespread icterus</td>
<td>acute nephritis, liver congestion</td>
<td>blood (days 2,3,4)</td>
</tr>
<tr>
<td>20383</td>
<td>ND</td>
<td>ND</td>
<td>kidney hypertrophy, liver discoloration, widespread icterus</td>
<td>haemorrhagic and necrotic pan-nephritis, liver autolysis</td>
<td>blood (days 2,3,4)</td>
</tr>
<tr>
<td>20912</td>
<td>ND</td>
<td>ND</td>
<td>kidney hypertrophy, liver discoloration, widespread icterus</td>
<td>haemorrhagic and necrotic pan-nephritis, liver autolysis</td>
<td>blood (days 2,3,4)</td>
</tr>
<tr>
<td>20342</td>
<td>ND</td>
<td>ND</td>
<td>kidney hypertrophy, liver discoloration, widespread icterus</td>
<td>haemorrhagic and necrotic pan-nephritis, liver autolysis</td>
<td>blood (days 2,3)</td>
</tr>
<tr>
<td>20913</td>
<td>RBC decrease, haematocrit decrease, (day 7)</td>
<td>urea increase, (day 7)</td>
<td>none</td>
<td>none</td>
<td>blood (days 2,3)</td>
</tr>
</tbody>
</table>

Table II. — Haematological, biochemical, macroscopic, histological and microbiological findings in control dogs after challenge with *Leptospira interrogans* serovar canicola.

ND: not done (dead before sampling day 7).
RBC: red blood cell.
before the end of the trial. Lesions detected during necropsy were similar in these 5 dogs and included a widespread icterus of mucosa, skin, subcutaneous tissues, surface of stomach, intestines, kidneys, liver and bladder; petechiae in cutaneous tissues, omentum, peritoneum, lung, mediastinal part of thorax and myocardium; ecchymosis and haemorrhagic areas on omentum, intestines, kidneys, liver and lungs and enlargement of kidneys. No abnormal gross lesions were observed in the surviving control dog and in the six vaccinated dogs which were necropsied at the end of the study (Table II). The proportion of dogs with gross lesions was significantly different between vaccinated group and control group \( P = 0.0152 \).

HISTOLOGICAL EXAMINATION

Four control dogs died during the first week PI (nos. 20916, 20383, 20912, 20342) and showed hepatic autolysis and severe haemorrhagic and necrotic pan-nephritis. One control dog (no. 20925) died later i.e. day 8 PI and developed a mild and diffuse congestion of liver and an acute interstitial nephritis with tubular dilatation (fibrino-haemorrhagic infiltrate) and tubular degeneration (atrophy of epithelium). Hepatic or renal lesions were not identified in the surviving control dog (no. 20913) and in treated animals (Table II). The proportion of animals with microscopic lesions was significantly different between groups \( P = 0.0152 \).

Discussion

The experimental inoculation with \( L. \) canicola can be considered as acute and severe since most of control dogs (83%) died within the first 8 days after the challenge. Only a high virulence of the inoculum can explain such a high mortality rate [13]. These 5 animals showed a similar clinical picture (fever, hypothermia, depression, anorexia, vomiting, abdominal pain, icterus, diarrhoea, dehydration, haematuria, weight loss). The day before its death (day 7), dog no. 20925 showed haematological abnormalities (leukocytosis, anaemia, platelet drop) and biochemical abnormalities i.e. a sharp increase in urea and creatinine (at least ten times the upper baseline values) which were closely related to the severity of the clinical signs. An acute renal failure can be considered as the cause of the death of this dog and also of the other ones by extension. The key role of the kidney failure in the fatal outcome was confirmed by the histological lesions which were an acute nephritis in dog no. 20925 and a necrotic pan-nephritis in 4 other dogs. At autopsy, all five dogs showed similar gross lesions (icterus, petechiae, haemorrhage, hypertrophy of kidneys, discoloration of liver). A leptospiromaemia was also seen during the first week PI in these 5 dogs. The surviving control dog showed a mild disease characterised by a weight loss, a leptospiromaemia and transient anaemia and urea increase. No significant changes were seen in macroscopic and macroscopic examinations. This surviving control dog showed evidence of greater innate resistance to the challenge exposure by reduced illness [4]. According to the same protocol (age and sanitary status of dogs, inoculum dose, administration route), the challenge with \( L. \) canicola induced the two opposed clinical pictures of the spectrum of Leptospirosis in unvaccinated dogs i.e. a mild disease and a severe syndrome of systemic infection with high mortality rate. Similar differences in clinical picture were already reported either after experimental inoculation with \( L. \) bataeviae [13] or with \( L. \) icterohaemorrhagiae [12] or after natural infection with \( L. \) sejroe [20, 19] or with \( L. \) canicola [6]. However such a high mortality rate was not recorded. In opposition, all vaccinated dogs showed neither significant clinical sign nor infection nor changes in laboratory parameters and such a quick and complete protection could be related to the high antigen mass of the canicola component (at least 800 millions bacterins per dose) in the vaccine [17]. The results also confirm that young unvaccinated dogs undergo greater risks of severe disease [18].

Two weeks after the second vaccine injection, all 6 dogs showed an early and significant individual MA antibody titre against \( L. \) canicola. Other similar leptospirosis vaccines were shown to induce few or undetectable antibodies after immunization of dogs [9, 4, 2, 15]. After challenge, the vaccinated dogs showed a humoral response increase which was however weaker than this observed in the control group. Although the serological results came from a single control dog, this pattern was already reported after experimental inoculation with \( L. \) canicola [14, 4, 2, 15]. Failure to isolate leptospira from blood of vaccinated dogs suggest a quick elimination of the challenge strain which should elicite a weaker serological memory response [4, 15].

The administration of the bivalent leptospirosis vaccine CANIGEN® L (twice at a 3 week interval) has been shown to provide complete prevention of leptospiromaemia, high mortality rate, clinical signs and gross and microscopic lesions after a virulent challenge with \( L. \) canicola. To our knowledge, it is the first time that such a protection is achieved in dogs by a commercially available leptospirosis vaccine.

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References


