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Original Article

Efficacy of a multivalent DAPPi-Lmulti canine vaccine against mortality, clinical signs, infection, bacterial excretion, renal carriage and renal lesions caused by *Leptospira* experimental challenges

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ABSTRACT

Efficacy of the *Leptospira* components of EURICAN[1] DAPPi-Lmulti, a canine combined vaccine, was tested after a primary course of two subcutaneous injections of one dose given 4 weeks apart to puppies aged from seven to nine weeks. Challenges with three pathogenic serovars of *Leptospira* spp. (Canicola, Icterohaemorrhagiae and Grippotyphosa) were carried out 14 days (onset of immunity studies – OOIs) and 13–15 months (duration of immunity studies – DOIs) after primary vaccination. During the four-week post challenge monitoring period, daily clinical observations were recorded, and blood (culture, biochemistry and haematology), urine (culture) and kidney (culture and histology) samples were collected regularly throughout the study or at necropsy. In OOIs, vaccination prevented mortality, clinical signs of the disease, infection, urinary excretion, renal carriage and renal lesions for all serovars. In DOIs, mortality, clinical signs and infection were less frequent in controls challenged with serovars Canicola and Grippotyphosa than in OOIs whereas they were absent or mild and transient in vaccinated dogs. Urinary excretion, renal carriage and renal lesions were at least significantly reduced in vaccinated dogs compared to controls for all serovars.

These studies demonstrated that the tested vaccine provides a quick onset of immunity as early as two weeks post-vaccination and a long-term immunity of 13–15 months with full protection against fatal leptospirosis for serovar Icterohaemorrhagiae; prevention of mortality and clinical signs, and reduction of infection, urinary excretion, renal carriage and renal lesions for serovar Canicola; and prevention of mortality and reduction of clinical signs, infection, urinary excretion, renal carriage and renal lesions for serovar Grippotyphosa.

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1. Introduction

Leptospirosis is one of the most widespread zoonotic disease in the world, affecting a broad range of mammals. It is caused by infection with pathogenic *Leptospira* spp. [1]. Clinical signs in dogs range from subclinical infection to acute disease [1–6]. Acute renal failure is currently predominant in symptomatic dogs, occasionally associated with hepatic injury [2,3,6,7]. Dogs recovering from leptospirosis may become asymptomatic carriers harbouring leptospires in kidney for extended periods, thus shedding leptospires into the environment, representing a zoonotic risk [1,7]. Infection of naive dogs results from contact with infected urine or urine-contaminated soil, water, food, or bedding [1,4]. Humans are incidental hosts and usually become infected through occupational, recreational or domestic contact with urine of carrier animals (such as the dog), either directly or via contaminated water or soil.

Recently, based on serological data, epidemiological situation on canine leptospirosis in Europe was updated and changes of *Leptospira* components in current dog vaccines were examined. It was concluded that the serovars Icterohaemorrhagiae and Canicola are still relevant, however other serovars such as Grippotyphosa and Bratislava should be considered [8].

In this paper we describe the level of protection demonstrated by onset and duration of immunity of a new canine vaccine containing *Leptospira* serovars Icterohaemorrhagiae, Canicola and Grippotyphosa. Two groups of dogs were challenged together with unvaccinated control dogs two weeks and 13–15 months after

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vaccination with heterologous strains of the three serovars challenged, and the effect of vaccination on mortality, clinical signs (including biochemical and haematological parameters), infection (*Leptospira* re-isolation from blood), urinary excretion (*Leptospira* re-isolation from urine), renal carriage (*Leptospira* re-isolation from kidney), and renal lesions was compared to control dogs in each study.

2. Materials and methods

Six separate vaccination-challenge studies were carried out to investigate onset and duration of immunity provided by EURICAN® DAPPi-Lmulti against three different pathogenic serovars of *Leptospira*: Canicola (Lc), Icterohaemorrhagiae (Li) and Grippotyphosa (Lg) (Table 1).

Each of the six studies had the same design, apart from the challenge material administered (serovar) and the time of this administration (OOI versus DOI) as indicated in Table 1. The sections below therefore describe shared procedures of all studies.

Trials were carried out in accordance with EU Directive 2010/63/EU for animals experiments. Institutional Animal Care and Use Committee approvals were obtained before conducting the studies. Endpoints were defined to avoid unnecessary suffering of diseased dogs.

2.1. Vaccines

EURICAN® Lmulti, a non-adjuvanted vaccine prepared from inactivated cultures of 3 *Leptospira* serovars (Canicola, Icterohaemorrhagiae and Grippotyphosa) at minimum protective dose, was used as diluent to reconstitute a non-adjuvanted freeze-dried vaccine, EURICAN® DAPPi, containing live attenuated canine distemper virus, canine adenovirus type 2, canine parvovirus type 2 and canine parainfluenza virus. An innovative and patented fedbatch process, performed in absence of bovine serum albumin in the culture medium [10], was used for the production of strains Li and Lg. It allowed a decrease of 40% of non-antigenic proteins per dose in EURICAN® Lmulti compared to bivalent formulation (EURICAN® L).

Puppies received subcutaneously two injections of EURICAN® DAPPi-Lmulti four weeks apart, first injection given at the age of seven to nine weeks.

2.2. Animals

Beagle puppies aged between seven and nine weeks without detectable agglutinating antibodies against the principal serovars of pathogenic *Leptospira* (Icterohaemorrhagiae, Canicola, Grippotyphosa, Serjoe, Hardjo, Hebdomadis, Pomona, Autralis and Autumnalis) were provided by a commercial supplier. Number of dogs per group an per study in presented in Table 1.

2.3. Challenge strains

The three challenge isolates were canine heterologous strains of the three serovars vaccinated. They were isolated from dogs. *Leptospira interrogans* serogroup and serovar Canicola strain Moulton and *Leptospira kirschneri* serogroup and serovar Grippotyphosa strain LG82 were obtained from National Centers for Animal Health (NCAH), Ames, Iowa, USA, and *Leptospira interrogans* serogroup and serovar Icterohaemorrhagiae strain 193 from Pasteur Institute, Paris, France. Identity of each strain was confirmed by National Reference Centre for *Leptospira* (CNRL Pasteur Institute, Paris, France) using molecular analysis.

Table 1
Experimental design.

Designation	Challenge		Group	Dogs #
	Challenge strain	Time after V2		
Onset of immunity (OOI)	Lc	2 weeks	V	7
			C	7
	Li	2 weeks	V	7
			C	7
	Lg	2 weeks	V	7
			C	7
Duration of immunity (DOI)	Lc	13 months	V	11
			C	10
	Li	15 months	V	10
			C	11
	Lg	13 months	V	12
			C	10

V: vaccinated group; C: control group.
V2: 2nd injection of primary vaccination.
Lc = *Leptospira interrogans* serovar Canicola.
Li = *Leptospira interrogans* serovar Icterohaemorrhagiae.
Lg = *Leptospira kirschneri* serovar Grippotyphosa.

2.4. Challenge suspension

After an initial culture in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium, the strain was back-passaged twice in hamsters to prevent loss of virulence through adaptation to culture conditions. After checking bacterial vitality and bacterial titer, each dog received 11 ml of challenge suspension with 0.5 ml instilled in the ventral conjunctival sac of each eye and the remainder administered intra-peritoneally. Inoculum per dog was between 7.7×10^8 and 6.4×10^9 organisms depending on the study.

2.5. Clinical examination

All dogs were observed daily for 28 days after challenge for signs consistent with leptospirosis. Six categories of signs (general condition, dehydration, ocular signs, vomiting, diarrheoa and cutaneo-mucosal signs) were daily evaluated. A scoring system was applied to each of these categories (see Table 2). Rectal temperatures were recorded daily for seven days after challenge. Dogs were weighed once a week.

Once first clinical signs appeared, clinical examination was performed twice a day and any dogs displaying serious and irreversible clinical signs that could lead to suffering were humanely euthanized based on predefined endpoints (hypothermia, shock, general condition score 2, vomiting or diarrheoa score 2 at three successive clinical examinations, daily score $\geqslant 9$).

2.6. Laboratory analyses

For serology, whole blood was collected before vaccination, before challenge and at the time of necropsy. Antibody titers against serovars Canicola, Icterohaemorrhagiae and Grippotyphosa were determined using microscopic agglutination technique (MAT) performed by standard operating procedures at a reference laboratory (CNRL). For the purposes of the studies, MAT titers 1:50 were considered positive.

For haematology (platelets count) and blood chemistry (urea, creatinine, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)), blood was regularly collected in heparin before and after challenge (see Table 3). Analyses were performed by ORBIO Laboratory (Bron, France).

For detection of Leptospirae by culture, blood (in heparin) and urine were regularly collected before and after challenge (see

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