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Research paper

A new tetravalent canine leptospirosis vaccine provides at least 12 months immunity against infection

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ABSTRACT

A key success factor in the vaccination of dogs against leptospirosis is long term protection against establishment of the renal carrier state, in order to protect other dogs, as well as humans, against this re-emerging zoonotic disease. In this paper, we describe the ability of a new European tetravalent vaccine containing antigen from *Leptospira interrogans* (*sensu lato*) serogroups Icterohaemorrhagiae, Canicola, Grippotyphosa and Australis to control infection and renal excretion in dogs at 12 months after vaccination.

In order to demonstrate the efficacy of all four vaccine components, four separate challenge studies were performed. For each study two groups of dogs were used (a group receiving the leptospirosis vaccine and a control group). Twelve months after the second vaccination all dogs in the vaccine and control groups were challenged, both intraperitoneally and conjunctivally, using a pathogenic challenge strain from one of four serogroups. Parameters recorded post-challenge were: clinical signs of disease, change in body temperature, total leucocyte count, thrombocyte count, presence of challenge organisms in blood, urine and kidney tissue, and evidence of interstitial nephritis at necropsy four weeks after challenge.

The vaccine was able to either prevent or significantly reduce infection following challenge with the strains of all four serogroups. The vaccine was also able to prevent or significantly reduce renal infection following Canicola and Icterohaemorrhagiae challenge, and there was a trend of reduction of renal infection with Australis (serovar Bratislava). In the case of the Grippotyphosa study, challenge led to no detectable renal infection in any dog of the control group.

In conclusion, in this study significant protective immunity was achieved in dogs 12 months after a basic vaccination schedule of two doses against strains of serogroups Canicola, Icterohaemorrhagiae, Grippotyphosa and Australis.

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1. Introduction

Although canine *Leptospira* vaccines are strictly regarded as 'non-core', many dogs are at risk of disease and thus vaccination is widespread in many countries. Traditionally, leptospirosis in dogs has been associated with serovars from serogroups Canicola and Icterohaemorrhagiae and bivalent vaccines containing strains from these serogroups have been used for the last 50 years. In recent years however there has been an increasing recognition of disease associated with serovars from other serogroups; in the USA from serogroups Grippotyphosa and Pomona and in Europe predominantly from serogroups Grippotyphosa and Australis (Ellis, 2010). As a consequence, in the USA, there are now a number of tetravalent canine leptospirosis vaccines available containing, in addition to traditional Icterohaemorrhagiae and Canicola antigens, strains from serogroups Grippotyphosa and Pomona. In Europe the vaccines are still predominantly bivalent, albeit with one recently-introduced trivalent product (serogroups Icterohaemorrhagiae–Canicola–Grippotyphosa). Most of the available vaccines, whether bivalent, trivalent or tetravalent, are regarded as being effective at controlling clinical disease and preventing mortality but only a few claim to be able to reduce infection or renal excretion following challenge; an important property in reducing the spread of this zoonotic disease (Feigin et al., 1973). Additionally, concerns have been raised about whether vaccine immunity persists for a full 12 months or whether more frequent re-vaccination is necessary.

A new European tetravalent vaccine containing antigen from *Leptospira interrogans* (*sensu lato*) serogroups Icterohaemorrhagiae, Canicola, Grippotyphosa and Australis has recently been developed (Nobivac® L4 – MSD Animal Health) which has been shown to reduce infection and/or renal excretion following challenge with specific serovars of these four serogroups shortly after vaccination (Klaasen et al., 2013). The following studies demonstrate the ability of this new vaccine to control infection and renal excretion in dogs at 12 months after vaccination.

2. Materials and methods

2.1. Animals

Six-week-old conventional beagle dogs without detectable agglutinating serum antibodies against *Leptospira* serogroups Canicola, Icterohaemorrhagiae, Grippotyphosa and Australis were provided by a commercial supplier. In each of the four studies, treatment groups (with nine dogs per group) consisted of pups of both sexes and pups derived from different litters in order to prevent gender and litter effects interfering with treatment effects. The selected dogs were free of clinical abnormalities or disease prior to inclusion in these studies. Husbandry was the same in each study; during the first part of the study (pre-challenge, up to 64 weeks of age) the dogs were housed in the dog facilities of the supplier; at the age of eight weeks the pups were weaned and for the challenge phase of the study the dogs were transferred to the animal facilities of MSD Animal Health, where, after being allowed to acclimatise for seven days, they were challenged at the age of 65 weeks. All housing systems used in these studies fully complied with the requirements of the Federation of European Laboratory Animal Science Associations (FELASA). The animal studies described in this paper were conducted after prior written approval by the responsible ethics review committee and thus this work follows international, national and institutional guidelines for humane animal treatment and complies with relevant legislation.

2.2. Study design

In order to demonstrate the efficacy of all four vaccine components, four separate challenge studies were undertaken using the same basic protocol. For each study two groups of dogs were used. One group (vaccine group) was vaccinated subcutaneously, twice with Nobivac® DHPPi* reconstituted in Nobivac® L4 at the ages of 6 and 10 weeks, and once (at the age of 6 weeks) with Nobivac® KC** intranasally. The second group (control group) was vaccinated twice with Nobivac® DHPPi reconstituted in Nobivac Solvent subcutaneously at 6 and 10 weeks of age, and once (at the age of 6 weeks) with Nobivac KC intranasally. Nobivac Solvent does not affect the immune response to the DHPPi vaccine, because it is a buffered salt solution, and Nobivac L4 licensing studies (in 2012 approved by the Committee for Medicinal Products for Veterinary Use of the European Medicines Agency) demonstrated that there was no effect of Nobivac DHPPi or Nobivac KC on the immune response in dogs to Nobivac L4. For the next year the dogs were housed under strict infection barrier conditions which prevented the possibility of exposure to field infection. Twelve months after the second vaccination all dogs in the vaccine and control groups were challenged, both intraperitoneally and conjunctivally, using a pathogenic challenge strain from one of four serogroups. Details of the grouping, vaccination schedules and challenge are shown in Table 1. For challenge with all four strains the method described in a recent publication (Klaasen et al., 2013) was used. However, to reduce the risk of a failing challenge, for the Bratislava strain two challenges on two consecutive days were performed.

2.3. Sample collection and parameters

Post-challenge the dogs were monitored for four weeks for any clinical signs of disease and change in body temperature. Samples of blood, serum and urine were collected at intervals during the four weeks following challenge and were evaluated for total leucocyte count, thrombocyte count and for the presence of challenge organisms or leptospiral DNA by culture and PCR, respectively (Klaasen et al., 2003; Ahmed et al., 2009). Four weeks after challenge the dogs were euthanized and a detailed post-mortem examination was undertaken. In addition a sample of kidney cortex was taken aseptically for leptospiral culture. In this study it was crucial to differentiate between dogs in which direct or indirect evidence was only found for leptospiraemia (early phase of the infection) and dogs in which renal infection (subsequent phase of the