Research paper

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

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A B S T R A C T

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 leptosiraemia (early phase of the infection) and dogs in which renal infection (subsequent phase of the
infection) was demonstrated. In accordance with the European Pharmacopoeia Monograph 0447 ("Canine Leptospirosis vaccine (Inactivated)") the somewhat arbitrary term infection (referring to the bacteraemic phase of the infection) and the term renal infection (referring to urinary tract infection and excretion) are used here to assess per challenge strain the numbers of dogs with infection and renal infection in vaccinated groups versus control groups. The following criteria were used to define a dog positive for infection and renal infection:

- **A dog positive for infection** (early stage of leptospirosis in which leptospiroma plays a central role) is a dog with at least two positive samples of blood or serum or urine/kidney on different days or a dog with challenge-induced nephritis or clinical or haematological evidence for leptospirosis. In general, an initial phase of bacteraemia (i.e., leptospiroma) has occurred in each dog in which infection, even in the absence of positive blood samples, or renal infection has been demonstrated, since: (i) challenge-induced clinical signs or haematological changes in the first two weeks are associated with bacteraemia, because these are effects of leptospires in the bloodstream (Faine, 1994a); (ii) in any dog in which challenge organisms are isolated from urine or kidney and in any dog with challenge-induced nephritis a preceding phase of bacteraemia has occurred: the bacteria are disseminated from the bloodstream, and not via another route, to the kidneys (Faine, 1994a, 1998).

- **A dog positive for renal infection** is a dog with at least one positive sample of urine/kidney from day 14 post-challenge onwards or challenge-induced nephritis (demonstrated by histopathological examination). This definition is considered valid, since: (i) the presence of leptospires in urine or kidney tissue from day 14 post-challenge onwards is considered as evidence for an active renal infection based on generally known scientific data on renal disease and patterns of urinary excretion in canine leptospirosis (Faine, 1998; Levett, 2001); (ii) challenge-induced nephritis is a result of ischaemia and inflammation caused by the prolonged presence of leptospires in the blood vessels of the kidney (Faine, 1994a; Levett, 2001). The numbers of dogs which were scored as positive for infection and renal infection in each of the four challenge studies were recorded and then compared to identify significant differences using a two-sided Fisher’s Exact test.

3. Results and discussion

Although in the USA serovar Bratislava has been reported as a pathogen in dogs (Adin and Cowgill, 2000), it is mainly in Europe where this serovar has been recognised as a dog pathogen (Ellis, 2010). In the present study the Bratislava strain was able to persist in the bloodstream of non-vaccinated control dogs for up to four days and to appear in the urine on days 3, 16 and 22 post-challenge, and caused interstitial nephritis in two control dogs (results not shown). These results clearly demonstrate the dog-pathogenicity of Bratislava.

The results are summarised in Table 2. As can be seen the vaccine was able to either prevent or significantly reduce infection following challenge one year after vaccination in all four studies. The vaccine was also able to prevent or significantly reduce renal infection following Canicola and Icterohaemorrhagiae challenge one year after vaccination. In the Canicola challenge study one out of nine vaccinated dogs had a positive urine culture on three out of five post-challenge sampling points, but without any evidence of leptospiral infection in the kidney (negative on renal culture and no signs of interstitial nephritis) at the end of the study. Nonetheless this dog was classified as "positive for infection" and "positive for renal infection" according to the criteria described. None of the other vaccinated dogs had any positive urine, whereas in all control dogs convincing evidence of renal infection was found (multiple positive urine cultures, interstitial nephritis). In the case of Australis challenge the difference between vaccinated and control dogs with renal infection was too small to reach statistical significance (P = 0.0824), due to a low number of positive control dogs (four out of nine control dogs). However, there was a clear tendency of reduction of urinary shedding by vaccination. With one additional control dog being positive the difference would have been statistically significant (P = 0.0294). Apart from the four positive control dogs, a fifth control dog had a positive urine sample on day 3 post-challenge but the other urine samples were negative, so that this dog was classified as negative for renal infection.
infection (results not shown). In the case of the Grippotyphosa study, challenge did not lead to detectable renal infection in any of the control group. In contrast, a similar challenge study in our laboratory using the same challenge (strain, method and dose) carried out in puppies at 13 weeks of age induced signs of renal infection in seven out of eight control dogs (Klaasen et al., 2013). Apart from the Bratislava study where a repeated challenge on two consecutive days was performed, it was observed that fewer control dogs had positive blood and or urine cultures in the present studies involving challenge in adult dogs than in the previously published studies with challenge in pups. This discrepancy can be explained by the fact that adult animals are known to have a higher resistance to infection with pathogenic Leptospira bacteria compared to young animals (Faine, 1994b). In the present Grippotyphosa challenge study there was also one vaccinated dog with signs of interstitial nephritis at post-mortem but with no evidence of the presence of leptospires (by either culture or PCR) and no clinical signs or evidence of thrombocytopenia (results not shown). Although this case, according to the definition of a dog positive for renal infection, has been scored as a positive case it is likely that the interstitial nephritis could have resulted from some other cause.

In these studies we were able to reproduce transient leptospiroemia and urinary shedding of the challenge organisms in non-vaccinated control dogs following challenge with pathogenic strains from serogroups Canicola, Icterohaemorrhagiae, and Bratislava, and transient leptospiroemia for Grippotyphosa. With this vaccine urinary shedding of leptospires is reduced, which implies that the vaccine helps prevent transmission of the infection to other animals and to humans and, therefore, is an aid in preventing these zoonotic infections (Feigin et al., 1973).

4. 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. Reduction of infection (leptospiroemia) was demonstrated against a Grippotyphosa strain, and reduction of infection, renal infection and urinary shedding was demonstrated against strains of serogroups Canicola, Icterohaemorrhagiae and Australis.

Conflict of interest

I am an employee of an R&D Department of MSD Animal Health (which is the name of Merck Animal Health in The Netherlands).

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