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Antibody response of dogs after immunisation with chimeric vaccine against borreliosis

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Abstract

Two chimeric recombinant fusion proteins (ch-rOspC and ch-rOspA) were created. They are composed of the immunodominant domains of OspC and OspA proteins described in the clinically most important strains of Borrelia. The gene constructs for these chimeric proteins were inserted into plasmids pET28 allowing induced gene expressions in a bacterial system.
The proteins were expressed in *E. coli* BL21 strains, purified and used for preparation of the vaccine.
One dose of the tested vaccines contained 50 µg of each relevant protein (ch-rOspC, ch-rOspA, or ch-rOspC+ch-rOspA). PET GEL A (Seppic) or Aluminium hydroxide gel as the immune adjuvants were used.
The dogs were vaccinated three times at 21 days intervals subcutaneously or intradermally and unvaccinated controls were also included.
The vaccine-elicited serum action antibodies specific to OspA and OspC were determined using in-house ELISA sets.
The immunisation induced specific antibody response in the vaccinated animals and OspC and OspA from representative genospecies *B. garinii*, *B. afzelii*, and *B. burgdorferi* sensu stricto were recognized. The control dogs were without antibody response.
ELISA examination enables determination of specific post-vaccination antibodies against OspA and OspC. Detection of these antibodies and their quantification may be used for evaluation of efficiency of vaccines.

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Keywords: Lyme disease; chimeric protein; antibody response; OspA; OspC

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1. INTRODUCTION

Lyme disease is a chronic multisystem infectious disease that is the most common arthropod-borne infectious disease both in Europe and in the United States. The disease is caused by a group of spirochetes collectively known as *Borrelia burgdorferi* sensu lato. This group of microorganisms is composed of three closely related, most pathogenic subspecies - *Borrelia burgorferi* sensu stricto, *Borrelia afzelii* and *Borrelia garinii*. While *B. burgdorferi* sensu stricto is the cause of virtually all Lyme diseases in North America, *B. garinii* and *B. afzelii* prevail in Europe[1].

The existing problems in diagnosis and treatment of Lyme disease and inability to effectively control and reduce the distribution of borrelia vectors gave rise to an urgent need to manufacture a vaccine that would be capable to effectively immunize susceptible species of domestic animals, especially dogs, against infection with *Borrelia burgdorferi* sensu lato[2, 3].

Vaccines based on the whole-cell bacterin *Borrelia burgdorferi* were developed for use in domestic animals. Other developed vaccines were based on the content of OspA and OspC proteins or other outer surface immunogenic proteins isolated from borrelia cultures, expressed as recombinant proteins in different hosts (*E. coli*) or prepared synthetically.

The contemporary experimental efforts are focused mainly on OspA and OspC antigens. The main reason is the possibility of using these antigens for vaccination purposes [4, 5, 6].

2. METHODS

Two chimeric recombinant fusion proteins (ch-rOspC and ch-rOspA) were created. They are composed of the immunodominant domains of OspC and OspA proteins described in the clinically most important strains of Borrelia. These purified proteins were used for preparation of the vaccine.

Table 1, Vaccination schedules

Vaccination schedule	Antigen		Immune adjuvants	Application	Vaccination Day 0	1st revaccination Day 26	2nd revaccination Day 42
1	rOspA+rOspC	50 µg + 50 µg	Aluxid	s.c.	Antigen	Antigen	Antigen
2	rOspA+rOspC	50 µg + 50 µg	Aluxid	i.d.	Antigen	Antigen	Antigen
3	rOspA+rOspC	50 µg + 50 µg	PET GEL A	s.c.	Antigen	Antigen	Antigen
4	rOspA+rOspC	50 µg + 50 µg	PET GEL A	i.d.	Antigen	Antigen	Antigen
5	-	-	Aluxid	s.c.	PLACEBO	PLACEBO	PLACEBO
6	-	-	Aluxid	i.d.	PLACEBO	PLACEBO	PLACEBO
7	-	-	PET GEL A	s.c.	PLACEBO	PLACEBO	PLACEBO
8	-	-	PET GEL A	i.d.	PLACEBO	PLACEBO	PLACEBO

The blood samples were taken in predetermined intervals and sera samples were prepared.

The post-vaccination antibodies against OspA and OspC in the sera samples were detected using in-house indirect sandwich ELISA sets with highly purified specific recombinant protein[7, 8].

Antibody levels in the tested sera were expressed as an OD_{450} values, compared to the values of the control positive and negative sera.

2.1 Substances for ELISA

- Antigen coating (rOspA *B. afzelii*, rOspA *B. garinii*, rOspA *B. burgdorferi* sensu stricto, rOspC *B. afzelii*, rOspC *B. garinii*, rOspC *B. burgdorferi* sensu stricto)
- Conjugate (Dog Immunoglobulin G (IgG) Goat anti-Dog Polyclonal (HRP) Antibody)
- Control positive antisera (dog origin)
  (anti *B. afzelii*, anti *B. garinii*, anti *B. burgdorferi* sensu stricto)
- Control negative serum (dog origin)
- Washing solution (PBS pH 7.2 + TWEEN 20)
- Blocking and dilution buffer (Skim milk + PBS pH 7.2 + TWEEN 20)
- Substrate (Sodium acetate buffer + TMB working solution + distilled water + H_2O_2 30%)
- Stop solution (H_2SO_4)