

RESEARCHES ON VICINAL IMMUNE RESPONSES IN CANINE LEPTOSPIROSIS

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Received July 6, 2011

ABSTRACT - Leptospirosis is a zoonosis of constant importance as causative microbial agent, maintained by subclinical infections in wild and domestic animals. Currently, for active immunization in dogs is used an inactivated vaccine, produced by specialized companies in different countries. The vast majority are polyvalent vaccines, which include the two leptospire serovars (*Leptospira canicola* and *Leptospira icterohaemorrhagiae*) and viral antigens for prevention of infectious hepatitis Rubarht, Carré's disease, coronavirus and parainfluenza. Specific immunity induced by this inactivated vaccine, is active, individual, installs in 10 to 21 days after administration, lasting a variable time (4-6 months) and never causes a 100% protection. This paper aims to investigate how this immune response in carnivores and its protection.

Key words: Immune response; *Leptospira icterohaemorrhagiae*; *Leptospira canicola*.

REZUMAT. Cercetări privind răspunsul imun post-vaccinal în leptospiroza

canină. Leptospiroza este o zoonoză de permanentă actualitate, întrucât agentul microbial cauzativ este menținut, prin infecții subclinice, la animale sălbatice și domestice. În prezent, pentru imunizarea activă a câinilor, sunt utilizate o gamă largă de vaccinuri inactivate, foarte eficiente, produse de firme specializate din diverse țări. În marea lor majoritate sunt vaccinuri polivalente, care includ, pe lângă cele două serovariante de leptospire, întâlnite frecvent la canide (*Leptospira canicola* și *Leptospira icterohaemorrhagiae*), și antigene virale pentru prevenirea hepatitei contagioase Rubarht, boala lui Carré, coronavirus și parainfluenza. Imunitatea specifică indusă de vaccin, inactivat este activă, individuală, se instalează în 10 - 21 de zile de la administrare, durează un timp variabil (4-6 luni) și niciodată nu determină o protecție de 100%. Prezenta lucrare are rolul de a investiga modul de instalare a răspunsului imun la carnivore, precum și protecția acestuia.

Cuvinte cheie : răspuns imun; *Leptospira icterohaemorrhagiae*; *Leptospira canicola*.

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INTRODUCTION

Leptospira infections represent an important health issue, causing endemic infections being transmitted to humans. Mortality in humans isn't high, but has very dramatic clinical manifestations (fever, toxic effect, headache, muscle pain, kidney damage, liver, nervous system, cardiovascular system) and is followed by a long convalescence (Blood *et al.*, 1989; VasIU, 2004; Velescu, 2002).

Leptospire are located at convoluted tubules level, and is excreted in the animal urine infecting the soil, water tanks, food, etc. (Perianu, 2003; Carter and Chenga, 1991; Constantinescu *et al.*, 1997). People get sick during bathing in ponds infected during agricultural works on wetlands, etc. Enter the body through wounds or erosions of the skin and mucous membranes.

Researches on the immune response after administration of different antileptospira vaccines, which included the two specific leptospire serovas (*Leptospira canicola* and *Leptospira icterohaemorrhagiae*) were taken in 2009 on three groups of 10 dogs, of different races, close in size and maintenance over the age of one year, belonging to paddock "B" Galați Municipality.

MATERIALS AND METHODS

The research was undertaken in 2009 on three groups of 10 dogs, of different races, close in size and

maintenance over the age of one year. For immunization were used commercial vaccines as: "Biocan L", "Nobivac LR", "Eurican DHPPI2LR". Before vaccines subcutaneously inoculation, the 30 dogs were subjected to blood collection and examined for the presence of specific antibodies to *Leptospira canicola* and *Leptospira icterohaemorrhagiae*.

To determine antileptospiric post-vaccination immune response from the three groups of dogs were collected blood samples 3 times every 10 days to 30 days and after 5 months after vaccine administration. Blood was harvested from saphenous vein, in sterile vacutainers and left at room temperature until the serum expression. Expressed serum was stored in a refrigerator until the execution of serological reactions.

For determination of specific antibodies, anti-*Leptospira canicola* and anti-*Leptospira icterohaemorrhagiae*, was used agglutination lysés reaction (RMAL), vs. eight leptospire serovas, maintained on Korthof environment.

Antigens represented by live cultures of leptospire (*Leptospira canicola* and *Leptospira icterohaemorrhagiae*), aged 6-12 days, incubated at 28 ° C in medium Korthof-looking uniform and a density of 100-600 micro-organisms on a microscope field (objective 20, ocular 15 Zeis) and not agglutinate spontaneously in the culture medium or in isotonic chloride solution. Cultures were checked before being used to a specific serum to see whether or not agglutinated.

The technique consists in performing the reaction a glass slide with a thickness of 2-2.5 mm. To this end were made serum dilutions, starting with the dilution 1 / 50. On clean glass slide and defatted in advance to put one drop, with easy heads of each dilution over to add a drop as far as possible equal to, the antigen (culture leptospire).

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Reaction was read under a microscope with dark background, after contact for 20-30 minutes at room temperature. Sera positive at first dilution 1 / 50, were further diluted (1/100, 1/200, 1/400, 1/800, etc.) until exhaustion titer.

RESULTS AND DISCUSSION

Serological researches regarding immune response, after inoculation of

the commercial vaccines on three test groups led to results depending leptospire serovar, vaccine structure and time since inoculation.

Results analytical interpretation shows an immune response at ten days from inoculation, translated through presence of antibodies for both serovars (*Table 1*).

Table 1 - Results antileptospiric immune response after 10 days of vaccines

No.	Vaccine	Leptospire serovar	Delivery number	Samples	Specific antibody titer							Medium titer
					1/ 100	1/ 200	1/ 400	1/ 800	1/ 1600	1/ 3200	1/ 6400	
1.	Biocan L	<i>L. i.h.</i>	1	10	1	3	4	1	1	0	0	1/570
		<i>L. canicola</i>	1	10	2	2	5	1	0	0	0	1/340
2.	NobivacLR	<i>L. i.h.</i>	1	10	2	3	3	1	1	0	0	1/360
		<i>L. canicola</i>	1	10	2	3	4	1	0	0	0	1/320
3.	Eurican DHPPI2LR	<i>L. i.h.</i>	1	10	3	3	2	2	0	0	0	1/330
		<i>L. canicola</i>	1	10	3	3	3	1	0	0	0	1/290

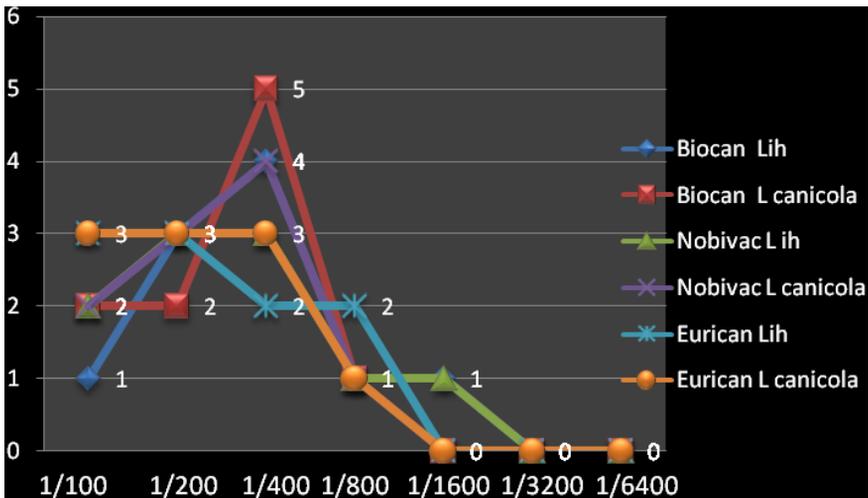


Figure 1 - Average titer values for the two leptospire

Considering results registered in *Table 1* it can be observed the fact that immune response intensity differs considering serovars. The medium titer differ depending leptospire serovar, being estimated between 1/330 and 1/570 for *Leptospira icterohaemoragiae* and 1/290-1/340 in *Leptospira canicola*.

From the point of view of the vaccine structure the highest titer value was obtained for *Leptospira icterohaemoragiae* 1/570 respectively 1/340 in *Leptospira canicola*, in samples harvested from the dogs inoculated with vaccine Biocan L, biological product that has only these two serovars in structure.

Decreasing antibody titer was obtained in samples from dogs inoculated with the vaccine "NobivacLR" with three antigens (two bacterial and viral antigen), followed by a medium titer, of 1/330 in *Leptospira icterohaemoragiae* and 1/290 in *Leptospira canicola*, in sera taken from dogs inoculated with "Eurican DHPPI2LR" vaccine which contains seven antigens (two bacterial and five viral).

Monitoring post-vaccination immune response, after 30 days of administration of three vaccines we have obtained the results recorded in *Table 2*.

Table 2 - Immune response at 30 days from vaccine inoculation

No.	Vaccine	Leptospire serovar	Delivery number	Samples	Specific antibody titer							Medium titer
					1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	
1.	Biocan L	<i>L. i.h.</i>	II	10	0	2	2	2	1	2	1	1/1720
		<i>L. canicola</i>	II	10	0	1	3	2	1	1	1	1/1420
2.	NobivacLR	<i>L. i.h.</i>	II	10	0	2	3	2	1	1	1	1/1440
		<i>L. canicola</i>	II	10	0	3	3	1	1	1	1	1/1220
3.	Eurican DHPPI2LR	<i>L. i.h.</i>	II	10	1	1	2	3	1	1	1	1/1115
		<i>L. canicola</i>	II	10	0	3	3	2	1	1	0	1/820

From results analyze is shown on the one hand an increase in the number of animals with high specific antibody value ranging from 1/800 and 1/6400 and on the other hand an increase in medium titer value for both serovars of the three biological products. Thus the average antibody titer values were between 1/1115 and 1/1720 for *Leptospira icterohaemoragiae* and

1/820 to 1/1420 for *Leptospira canicola*.

Average titer values differ depending on vaccine used. Thus, the highest value of average titer of 1/1720 to *Leptospira icterohaemoragiae* and 1 / 1420 to *Leptospira canicola*, were obtained for vaccine "Biocan L", and the lowest average titer values were

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obtained in *Leptospira serovars icterohaemorrhagiae* of 1 / 1115 and *Leptospira canicola* 1 / 820, contained

in "Eurican DHPPI2LR" structure (Fig. 2).

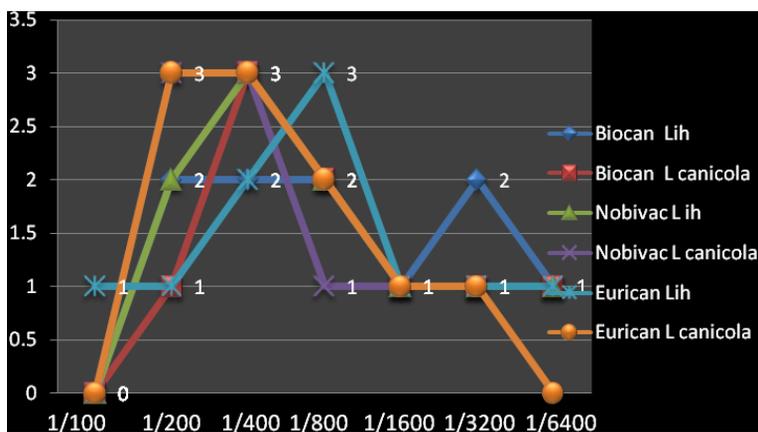


Figure 2 - Average titer values after 30 days from inoculation

Table 3 - Immune response after 5 months from immunization

No.	Vaccine	Leptospire serovar	Delivery	Samples	Antibody titer						Average titer	
					1/100	1/200	1/400	1/800	1/1600	1/3200		1/6400
1.	Biocan L	<i>L. i.h.</i>	III	10	1	3	3	3	0	0	0	1/430
		<i>L. canicola</i>	III	10	2	4	3	1	0	0	0	1/290
2.	NobivacLR	<i>L. i.h.</i>	III	10	3	3	3	1	0	0	0	1/290
		<i>L. canicola</i>	III	10	3	4	2	1	0	0	0	1/270
3.	Eurican DHPPI2LR	<i>L. i.h.</i>	III	10	4	3	2	1	0	0	0	1/260
		<i>L. canicola</i>	III	10	5	3	1	1	0	0	0	1/230

From results shown in Table 3, regarding immune response after 5 month, appears a rise in animal number with antibody titer values between 1/100-1/400, and on the other hand a decrease in medium titer value depending leptospire serovar. The highest values between 1/320 -1/430 were registered for *Leptospira*

icterohaemorrhagiae and 1/230 - 1/290 for *Leptospira canicola*.

Average titer values are different depending on the antigenic structure of the vaccine inoculated. Thus the largest value of the average titre of 1/430 for *Leptospira icterohaemorrhagiae* and 1/290 to *Leptospira canicola* has been obtained for the vaccine "Biocan L". Values less than 1/290 for *Leptospira*

icterohaemorrhagiae and 1/290 to *Leptospira canicola* have been obtained for the vaccine "NobivacLR" and the lowest values of average titer

of 1/260 -1 / 230, were obtained using vaccine "Eurican DHPPI2LR" (Fig. 3).

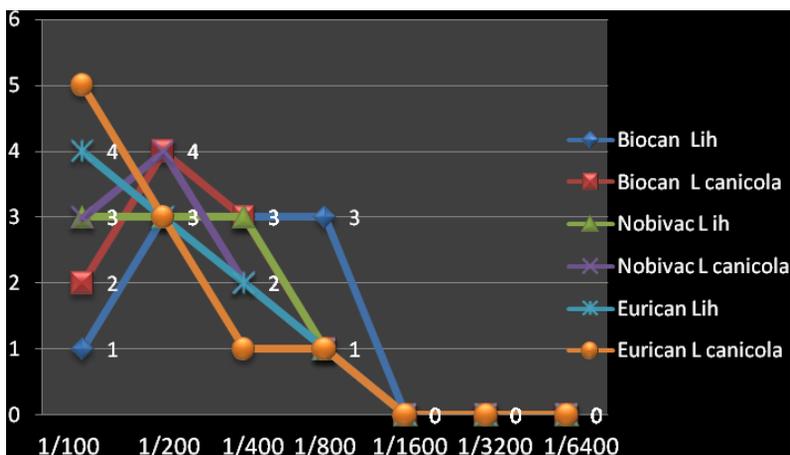


Figure 3 - Average titer values after 5 months from immunisation

Analyzing the immune response dynamics at 10, 30 days and 5 months after administration of the vaccines, there is, on the one hand, an increase in the average titer after 30 days for both leptospire serovars (*Leptospira icterohaemorrhagiae* and *Leptospira canicola*), included in the vaccines structure, compared with values obtained from the examination after 10 days and on the other hand, a decrease in the average titer after 150 days from immunization.

Increased average titre value after 30 days after inoculation of the biological products, explains antigenicity and post-vaccination immunity. Lower average titer after 5 months post-vaccination explains the decrease of protection.

In fact, it is known that the immune response and therefore post-vaccination immunity is dependent as intensity and duration on nature of antigen (bacterial or viral, inactivated or attenuated).

Average titer progressively drop after 5 months from immunisation, can be explained by the fact that the duration of immunity obtained using inactivated bacterial antigens, is shorter, being between 4-6 months.

CONCLUSIONS

Results obtained shows that after immunisation with three biological products, all animals exhibited immune response, by the appearance of specific antibodies in blood serum.

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The intensity of immune response, and average titer values differ depending on the structure of *Leptospira serovar* present in the three types of vaccines, ranging from 1 / 420 - 1 / 1720 for *Leptospira icterohaemorrhagiae* and *Leptospira canicola* 1/230-1/1420.

Average titer values are different depending on the antigenic structure of the vaccine. The highest value of average titer of 1 / 430 for *Leptospira icterohaemorrhagiae* and 1 / 290 to *Leptospira canicola* was obtained from the vaccine "Biocan L".

Values of 1 / 290 for *Leptospira icterohaemorrhagiae* and 1 / 290 for *Leptospira canicola* have been obtained for the vaccine "NobivacLR", and the lowest average titers of 1 / 260 and 1 / 230 were obtained for "Eurican DHPPI2LR".

Analyzing the immune response dynamic at 10, 30 days and 5 months, it appears, on the one hand, increased value of the average titer after 30 days in both leptospire serovars, compared with values obtained after 10 days, on the other hand, decreased antibody average titer after 5 months from immunisation.

Average titre increased value after 30 days from inoculation of the biological products highlights antigenicity of the vaccine strains in the product structure, post-vaccination immunity. Lower average titer after 5 months shows post-vaccination protection decrease of immunity.

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