

# PRODUCTION OF A POLYCLONAL ANTIBODY AGAINST *LEISHMANIA INFANTUM*

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## INTRODUCTION

### OBJECTIVE

PRODUCE A POLYCLONAL ANTIBODY TO BE USED IN THE IMMUNOHISTOCHEMICAL DIAGNOSIS OF CANINE LEISHMANIOSIS (CanL).

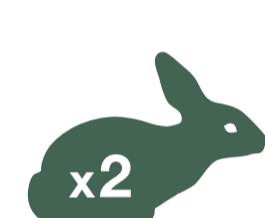
### HYPOTHESIS

Immunization of rabbits with a commercial vaccine against canine leishmaniasis (CaniLeish®) would be able to induce a **specific polyclonal immune response** and therefore generate a serum usable in immunohistochemical techniques.

## MATERIALS AND METHODS

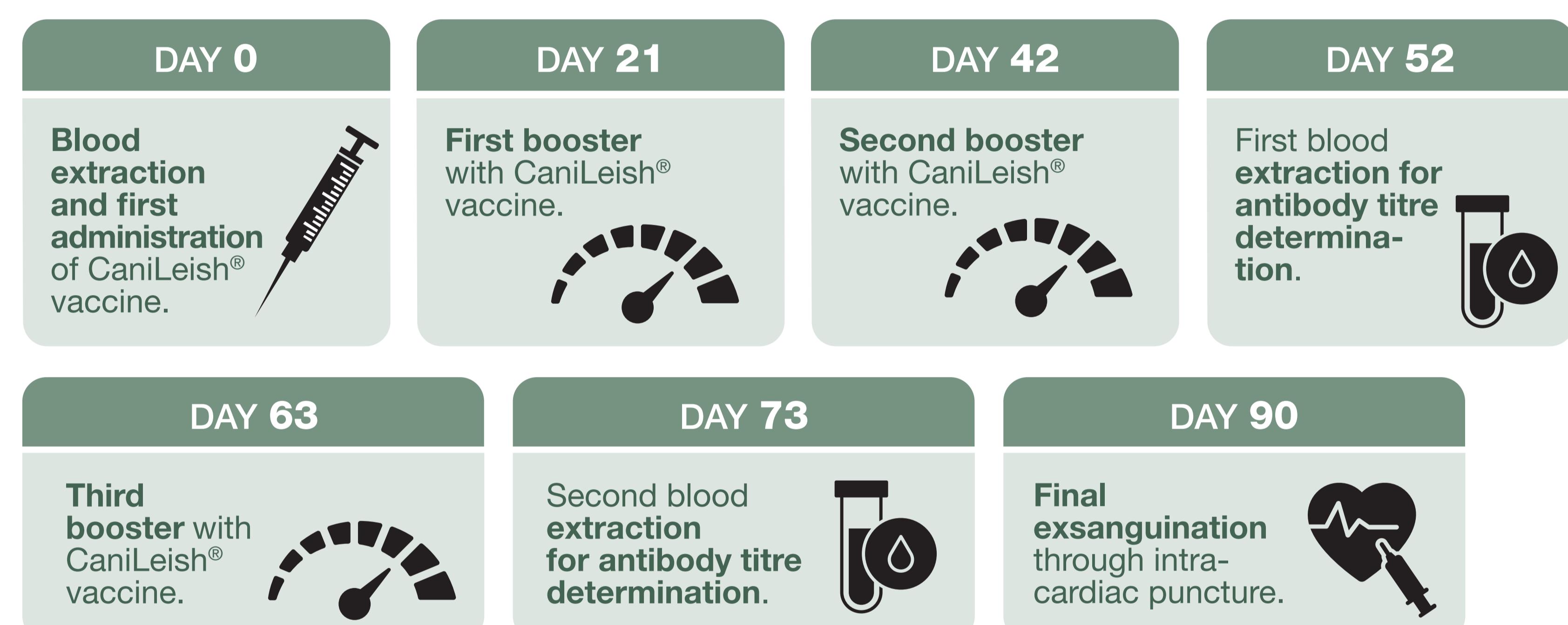
### 1

#### POLYCLONAL ANTIBODY PRODUCTION IN RABBITS



Two New Zealand White female rabbits of 2 kg each were used

#### EXPERIMENTAL IMMUNIZATION



### 2

#### TITULATION OF ANTIBODIES AGAINST LEISHMANIA

It was performed through a **ELISA**

Start time:	10/28/19	12:34:53				
<>	1	2	3	4	5	6
A	2,2133	1,2657	0,7535	0,4789	0,2613	0,1824
B	0,1211	0,095	0,0759	0,0719	0,0636	0,0598
C	2,8589	1,5226	0,9454	0,4844	0,2669	0,1682
D	0,1163	0,0884	0,0756	0,0688	0,0642	0,0652
E	0,0717					
F	0,1126					
G	0,06					
H	0,0567					

Results:  
Both rabbits produced a high specific IgG antibody titre to *Leishmania infantum*. The titres were similar in both sera, although in the second one (1831CK1) it was somewhat higher.

### 3

#### IMMUNOHISTOCHEMISTRY

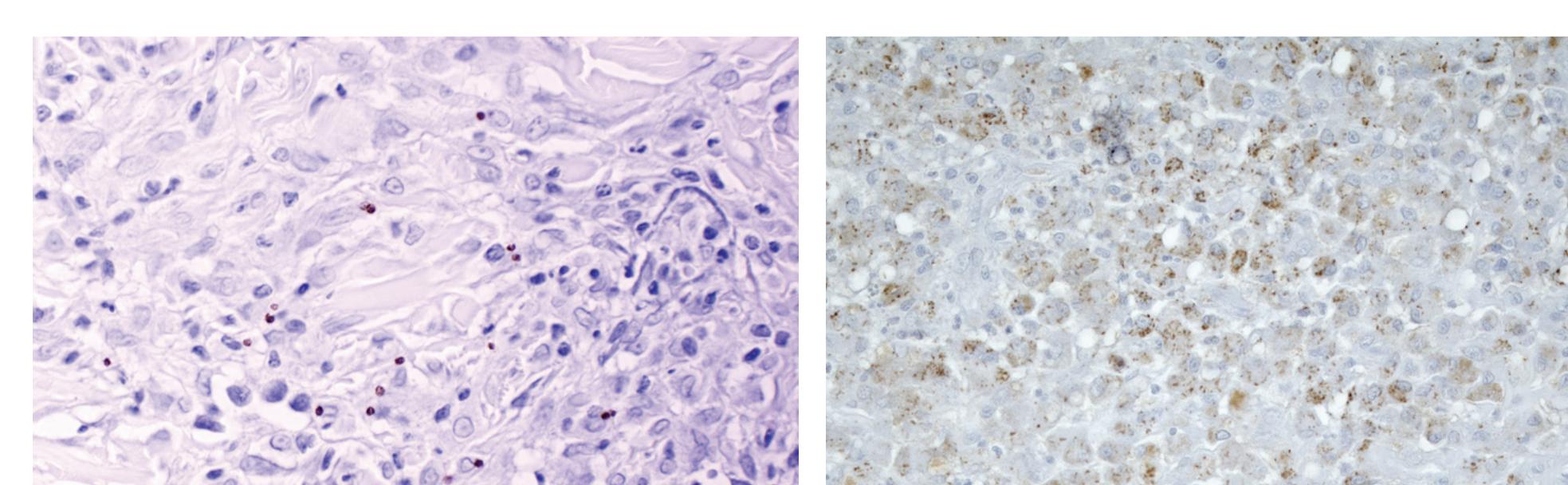
Paraffin sections of **two confirmed cases** of leishmaniasis (a lymph node and a skin section) were used to test the antibodies.

The two sera were used as a **primary antibodies** in a Peroxidase-Antiperoxidase technique (PAP) at a 1:1000 dilution.

Sections were **depigmented** with potassium permanganate and oxalic acid and **counterstained** with haematoxylin.

The results were **compared** with those obtained with a polyclonal antibody of reference.

**RESULTS:** the results were satisfactory given the **big amount of stained amastigotes**. However, the stained sections showed a strong background and it was concluded that a **purification of the antisera** was necessary.



Immunohistochemical detection of *Leishmania*

## CONCLUSIONS

Our hypothesis was **confirmed**. The immunization of two rabbits with a commercial vaccine against *leishmaniasis* (CaniLeish®) has been **able to induce a specific polyclonal immune response**. Both sera showed a **high titer** of anti-*Leishmania* antibodies when tested with an ELISA. This can be a useful approach to produce specific anti-*Leishmania* antisera.

## BIBLIOGRAPHY

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