

Centrin gene-deleted *Leishmania major* vaccine protects dogs against canine visceral leishmaniasis following three natural exposures to infected sandflies in Northern Tunisia

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SUPPLEMENTARY DATA

SUPPLEMENTARY FIGURE

Supplementary Figure 1. Kinetic of IgG anti-SLA. Levels of IgG specific of soluble leishmanial antigen (SLA) were measured in sera from vaccinated dogs (a) and those from control group (b) before exposure and at return from the field after each exposure period.

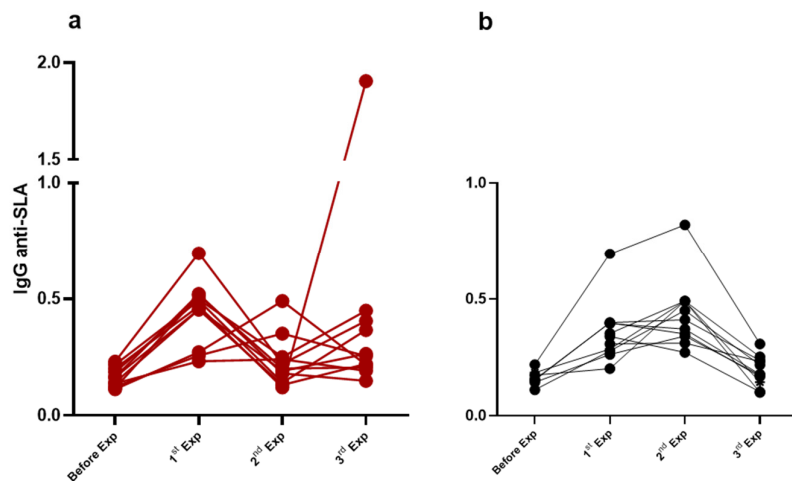
Supplementary Figure 2. Immune response in the spleen of dogs after three exposures in *L. infantum* endemic area. Individual results of the fold-increase ($2^{-\Delta\Delta CT}$ method) of (a) IFN- γ , (b) IL-10 and (c) TNF- α mRNA expression levels within spleen aspirates from *LmCen*^{-/-} vaccinated dogs and those from the control group (PBS) using Taqman qRT-PCR. Horizontal bars correspond to median value for each group. Statistical analysis was done using non-parametric Mann Whitney U test (* $p < 0.05$).

SUPPLEMENTARY TABLE

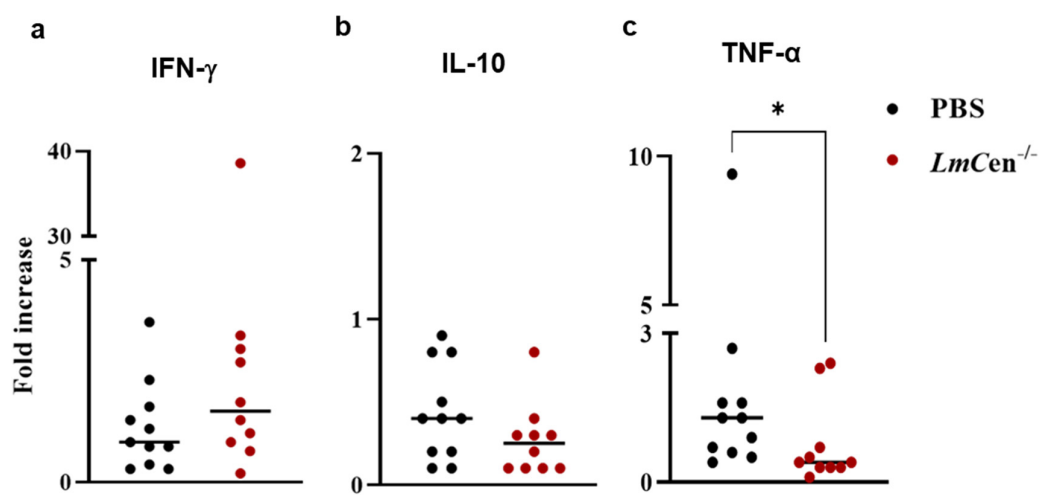
Supplementary Table 1. Sequences of primers and probes used to measure cytokines mRNA expression levels in dogs' spleen.

Supplementary Table 2. Serum biochemical and hematological profile in dogs enrolled in the study at 15 days post-prime. Biochemical analyses at 15 days post-vaccination showed increased urea concentration and a mild to moderately increased liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Changes in hematological parameters include leukocytosis, lymphocytosis, and an increased hemoglobin concentration, which can be linked to the stress at the time of blood collection.

Supplementary Table 3. Clinical examinations using score (CS) (Sum of points= SC). Impact of the vaccination of dog with *LmCen*^{-/-} after the 3rd exposure in the endemic area for transmission of *L. infantum* in Tunisia.



Supplementary Figure 1.



Supplementary Figure 2.

Supplementary Table 1. Sequences of primers and probes used to measure cytokines mRNA expression levels in dogs' spleens.

Target gene	Primers & Probes	Sequence (5_→3_)
IFN- γ	F	TCAACCCCTTCTCGCCACT
	R	GCTGCCTACTTGGTCCCTGA
	P	CCCCACCCGAACCTCTTCCT
TNF- α	F	ACCCATGTGCTCCTCACC
	R	AGGGCTCTTGATGGCAGAGA
	P	CGCTTCGCCGTCTCCTACCA
IL-10	F	CGACCCAGACATCAAGAACC
	R	CACAGGGAAGAAATCGGTGA
	P	TCCCTGGGAGAGAAGCTCAAGACCC
β -actin	F	GACCCTGAAGTACCCATTGAG
	R	TTGTAGAAGGTGTGGTGCCAGAT
	P	CGTCACCAACTGGGACGACATGGA

Supplementary Table 2. Serum biochemical and hematological profile in dogs included in the immunogenicity study at 15 days post-prime.

	Urea	Creatinine	Total proteins	AST	ALT	GGT	Red blood cells	Hemoglobin	Hematocrit	MCV	MCH	MCHC	White blood cells	PNE	Lymphocytes	Neutrophils	Monocytes	Thrombocytes
Usual values	3,3-6,6 mmol/l	60-130 μmol/L	54-71 g/l	6-13 IU/l	5-25 IU/l	2-10 IU/l	5,3-8,3 10 ¹² /l	12-18 g/dl	37- 55 %	60- 77 fl	20-25 pg	32 – 36 g/dl	6 – 18 10 ⁹ /l	2-10 %	12 à 30%	60-75%	3 à 10%	200-500 10 ⁹ /l
Dog code																		
4452	8,83	65	66,6	52	42	5	7,93	19,1	52,2	65,8	24,1	36,6	13620	0,2	26	73,8	0	400
4454	10,33	85	70,7	61	35	5	7,19	17,9	48,5	67,5	24,9	36,9	12520	0,3	37,3	62,4	0	435
4458	10,83	94	66,8	67	30	3	6,73	15,6	44,6	66,3	23,2	35	14010	0,3	34,7	64,8	0	471
4471	6,83	65	66,6	47	36	4	7,18	16,8	47,1	65,6	23,4	35,7	10870	0,5	28	71,4	0	401
4455	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4485	11	79	71,5	61	42	3	7,7	18,4	48,9	63,5	23,9	37,6	14090	0	28	71,8	0,1	383
4486	10,33	70	68,3	53	49	2	6,59	16,4	45,1	68,4	24,9	36,4	18720	0	28	72	0	274
4488	6,17	70	65,3	63	52	1	7,44	17,8	49	65,9	23,9	36,3	18240	0	25,3	74,6	0	446
4469	7,83	74	62,2	61	32	5	7,37	15	42,1	57,1	20,4	35,6	14600	0	53,6	45,9	0,2	265
4473	6	66	65,8	33	34	5	7,58	18,5	49,8	65,7	24,4	37,1	13460	0,6	26	73,2	0,1	448
4475	8,33	70	61,2	48	36	0	7,57	18	50,6	66,8	23,8	35,6	13190	0,1	43,5	56,4	0	493
4494	8,17	70	67,9	49	48	8	7,74	19,8	53,9	69,6	25,6	36,7	12280	0,1	30,5	69,4	0	413

AST: Aspartate aminotransferase, **ALT:** Alanine aminotransferase, **GGT:** Gamma-glutamyl transferase, **MCV:** Mean corpuscular volume, **MCH:** mean corpuscular hemoglobin, **PNE:** polynuclear neutrophils. Control group, dogs received 100μl of medium (Green), dogs received an injection of 10⁶ *LmCen*^{-/-} (Red), and dogs received an injection of 10⁷ *LmCen*^{-/-} (Blue).

Supplementary Table 3. Clinical examinations using score (CS) (Sum of points= SC). Impact of the vaccination of dog with *LmCen*^{-/-} after the 3rd exposure in the endemic area in the endemic area for transmission of *L. infantum* in Tunisia.

Category	Hair	Weight	Nails	Lymph nodes	Oral mucosa	Conjunctivitis	Spleen	Total
	Score (0, 1, 2)							
PBS	Dog1*	2	0	2	0	0	0	4
	Dog2*	0	1	0	0	0	0	1
	Dog3	0	0	0	0	0	0	0
	Dog4	0	0	2	1	0	0	3
	Dog5*	2	1	2	1	0	0	6
	Dog6*	2	1	0	1	0	0	4
	Dog7	2	1	0	1	0	0	4
	Dog8	0	0	0	0	0	0	0
	Dog9	0	0	2	0	0	0	2
	Dog10	0	0	0	0	0	0	0
	Dog11	0	0	2	0	0	0	2
<i>LmCen</i> ^{-/-}	Dog1	0	1	2	0	0	0	3
	Dog2	0	0	2	0	0	2	4
	Dog3	0	0	0	0	0	0	0
	Dog4*	2	2	2	2	1	2	13
	Dog5	0	0	2	0	0	0	2
	Dog6	0	0	0	0	0	0	0
	Dog7	0	0	0	0	0	0	0
	Dog8	0	1	0	0	0	0	1
	Dog9	0	0	2	0	0	0	2
	Dog10	0	0	0	0	0	0	0
	Dog11	2	1	0	0	0	0	3

* Leishmaniasis was confirmed by the parasites' detection in spleen aspirate or blood