Typical Leishmaniosis in a Dog Regularly Vaccinated with Canileish®

Alessandra Gavazza, Anyela A. Medina Valentin, George Lubas*

Abstract
The vaccine Canileish® is distributed in Europe to reduce the risk of developing an active infection and clinical leishmaniosis. An English Setter dog vaccinated with Canileish® and treated with anti-feeding and repellent medications showed typical clinical signs of leishmaniosis. The dog was presented with dysorexia, weight loss, fever and forelimb lameness. The physical exam revealed moderate generalized external lymph node enlargement, sero-purulent ocular discharge, photophobia, and swollen and painful right carpal joint. Clinico-pathological findings revealed moderate microcytic-hypochromic non-regenerative anemia, mild neutropenia and thrombocytopenia, hyperglobulinemia, hematuria and mild elevation of urine protein-to-creatinine ratio, polyclonal peak in the gamma globulins, *Leishmania* spp. amastigotes in lymph nodes and bone marrow, and immunofluorescence antibody titer (IFAT) of 1:5120. The successful treatment included meglumine antimonate and allopurinol for 40 days, and metronidazole-spyramicin for 24 days. The dog was monitored up to 9 months and normalization of most hemato-biochemical abnormalities was achieved. The bone marrow qPCR for *Leishmania infantum* was negative, while IFAT was 1:160. Despite the systematic leishmaniosis prevention, the typical clinical disease can occur.

Keywords
leishmaniosis — dog — Canileish® — clinico-pathological findings — treatment

Dept Veterinary Sciences, University of Pisa, Via Livornese Lato Monte, San Piero a Grado 56122, Pisa, Italy
***Corresponding author**: george.lubas@unipi.it

Introduction

A vaccine for canine leishmaniosis (Canileish®) has been introduced in several European countries including Italy[1], starting from 2011. Its formulation origins from LiESAp (culture supernatant of *L. infantum promastigotes*) and the 54-kDa excreted protein of *Leishmania infantum* in addition to muramyl dipeptide (MDP) as adjuvant. The vaccine is designed to stimulate an active immunity (cell-mediated) in *Leishmania* sero-negative dogs in order to reduce the risk of developing an active infection (12% in vaccinated vs. 33% in control dogs) and related clinical disease (7% vs. 23%) after the exposure to *L. infantum*. The overall coverage is expected to reach 68% (2, 3, 7, 8, 9). Recently, a case series of sixteen dogs affected by clinical leishmaniosis previously vaccinated with Canileish® have been reported as an Abstract (10). Here, a case of a dog regularly immunized with Canileish® and treated with anti-feeding and repellent medications presenting with a severe form of leishmaniosis, is described.

Material and Methods

"Alba", an English setter dog, intact female of about 4 years old was referred to the authors' facility for dysorexia, weight loss, fever and forelimb lameness (beginning of March 2015). "Alba" was living with 2 other dogs (both negative on *Leishmania* IFAT, Indirect Fluorescence Antibody test and conjunctival PCR, Polymerase Chain Reaction) in Northern Tuscany (Italy), and was used for hunting. "Alba" was regularly vaccinated since the age of 3 months with vaccines against distemper, viral hepatitis, adenovirus, and leptospirosis[2]. Furthermore, from the age of 9 months she was regularly vaccinated against *Leishmania* using Canileish® according to the manufacturer's instruction. Before the vaccination with Canileish®, "Alba" underwent a complete physical examination and partial hemato-biochemical analysis including a Speed-Leish K® test. Following the manufacturer's instruction on Canileish®, prophylaxis against ectoparasites was concurrently applied (repellent and anti-feeding, imidacloprid and permethrin[3], heartworm prevention with moxidectin[4]). After the admission (T0) a follow-up period of 9 months at different days (T6, T10, T24, T42, T85, T148, T265) for "Alba" was performed.

At physical examination the body condition score was 2/9 (Figure 1 and 2) and the temperature was 39.6°C. In addition, moderate generalized peripheral lymph node enlargement (Figure 3 and 4), sero-purulent ocular discharge with photophobia (Figure 5) and swollen and painful right carpal joint (Figure 6), were recorded. Complete blood count (CBC) including the stained blood smear evaluation, serum biochemical profile (including total calcium, phosphates, iron, total proteins, albumin, fructosamine, C-reactive protein, urea, creatinine, total bilirubin, cholesterol, triglycerides, glucose, alkaline phosphatase, gam-

[1]Canileish; VirbacS.r.l., Milan, Italy (A.I.C., Italian marketing authorization, #104281062)

[2]Nobivac CEPPi+L; Intervet Italia S.r.l., Peschiera Borromeo, MI, Italy

[3]Advantix; Bayer Animal Health, Milan, Italy

[4]Guardian Sr injectable; Eli Lilly Italia S.p.a., Sesto Fiorentino, FI, Italy

Figure 1. Body condition score 2/9 from the rear view at T0

Figure 2. Body condition score 2/9 from the front view at T0

maglutamyl transferase, aspartate amino transferase, alanine amino transferase, creatin phosphokinase, lactate dehydrogenase, amylase, sodium, potassium, chloride and bicarbonate), coagulation profile (including prothrombin time, partial thromboplastin time, and fibrinogen), serum protein electrophoresis (separation on albumin, alpha 1 and 2 globulins, beta 1 and 2 globulins, and gamma globulins), serology for *L.infantum* (cut-off value: negative for exposure, 1:160 for possible disease), complete urinalysis including UP/C ratio, fine needle aspirations for cytology of prescapular and popliteal lymph nodes and aspiration of bone marrow, were performed. A sample of bone marrow aspirate was also submitted for *Leishmania* quantitative PCR (qPCR).

Results

The presenting physical complaints resolved at different times: body weight progressively gained and normalized at T42, fever lasted for 10 days, photophobia disappeared by day 10 but blepharitis occurred at T3 (Figure 7 and 8), persisted at T10 (Figure 9), and disappeared at T24 (Figure 11), sero-purulent ocular discharge decreased over 15 days, lymph nodes were almost normal by T24, and forelimb lameness disappeared by T3. It should be noticed that at T10 a large ulcer at the tail was evidenced (Figure 10), but almost completely recovered at T24 (Figure 12).

The CBC initially showed a moderate microcytic-hypochromic non-regenerative anemia (Hematocrit, Hct 23.9%, reference ranges, RR, 37.3-61.7%; Mean Corpuscular Volume, MCV 54.7 fl, RR 61.6-73.5; Mean Corpuscular Hemoglobin Content, MCHC 35.6 g/dL, RR 32.0-37.9; Mean Corpuscular Volume, MCH 19.5 pg, RR 21.2-26.9; reticulocytes 17.0 K/μL, RR 10-110), mild neutropenia (3.0 K/μL, RR 3.7-11.9), normal lymphocyte count with reactive lymphocyte and mild thrombocytopenia (132 K/μL, RR 148-484). Starting from T3, the microcytic hypochro-mic RBCs slowly returned to normal by T85, the neutrophil count normalized by T10, the reactive lymphocytes disappeared from blood smears by T85 and the platelet count normalized by T10. The CBC was normal at T148 although few nucleated RBCs and Howell-Jolly bodies were observed. At T265 the CBC was within the reference ranges for all parameters.

The biochemical profile showed, initially, severe hyperproteinemia (11.5 g/dL, RR 5.8-7.8) (normal at T85) with low albumin (1.9 g/dL, RR 2.6-4.1) (normal at T42), hyperglobulinemia (9.7 g/dL, RR 2.5-4.5) and low albu-

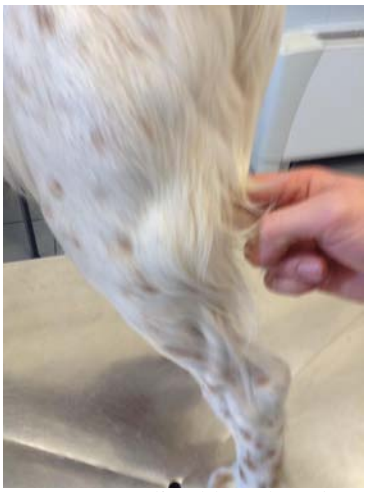

Figure 3. Popliteal lymph node enlargement at T0

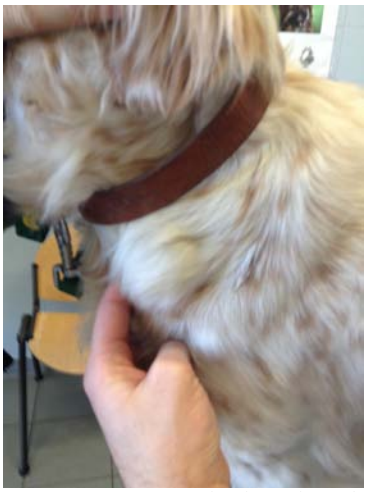

Figure 4. Prescapular lymph node enlargement at T0

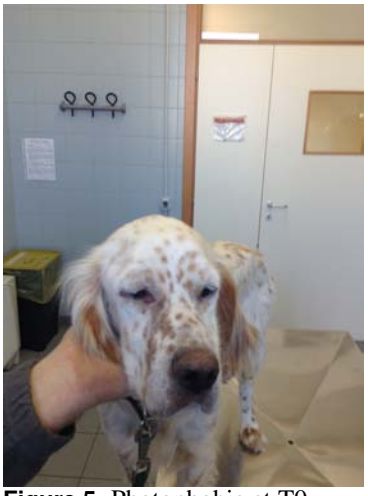

Figure 5. Photophobia at T0

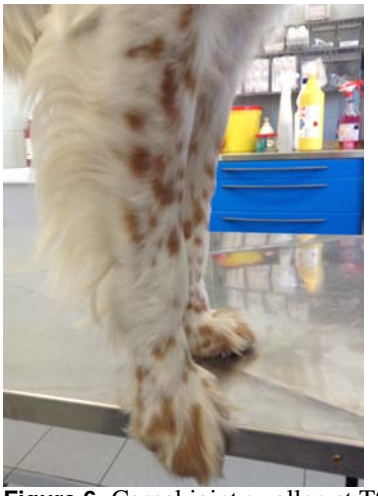

Figure 6. Carpal joint swollen at T0

min/globulins ratio (0.2, RR 0.5-1.5) (both normal at T85), elevated C-reactive protein (2.3 mg/dL, RR 0.0-0.3) (normal at T24), slight increase of AST (75 U/L, RR 15-40) and LDH (338 U/L, RR 20-160) (normal at T24). Then, from T42 the biochemical profile was unrewarding and all parameters were within the reference ranges.

The coagulation profile was investigated at T0, T27, T148 and T265, and was always normal during the disease course.

The serum protein electrophoresis at T0 (Figure 13A) showed hypoalbuminemia (22.2%) and a polyclonal peak in the gamma globulins (55.2%) that returned slowly to normal at T148 (Figure 13B). At T265 this assay was unremarkable.

The urinalysis initially showed hematuria (normal at T6) and mild elevation of urine protein-to-creatinine ratio (0.64, RR 0.1-0.5) (normalized at T85). In successive samplings all the urine parameters were within the references ranges.

At admission (T0) the lymph-node aspirates showed lympho-plasmacellular hyperplasia with *Leishmania* amastigotes extracellularly and in the macrophages; further aspirations were not performed because the lymph nodes were almost not palpable by T42. The bone marrow aspirate at T0 yielded mild hyperplasia of myeloid series (as compared to erythroid series), and slight infiltration with plasmacells and macrophages showing *Leishmania* amastigotes. The bone marrow aspirate at T148 yielded mild dysplasia in the myeloid series and slight prevalence of erythroid cells (as compared to myeloid). At T265 the bone marrow evaluation was normal.

Quantitative PCR (qPCR) performed on bone marrow samples was negative for *Babesia* spp, *Bartonella* spp, *Ehrlichia canis*, *Hepatozoon* spp, and *Rickettsia* spp but extremely positive for *L. infantum* (1,212,000,000 copies). The bone marrow qPCR, repeated at T148 and T265, was negative for *L. infantum*. At admission (T0) IFAT titer was extremely high (1:5120) and the Speed-Leish K® assay for *Leishmania* spp. was positive. The IFAT titer was reduced at T148 (1:320) and T265 (1:160) whereas the Speedy-Leish K® test was still positive.

"Alba" was treated with a combination of meglumine antimonate[5] (subcutaneous, SC) and allopurinol[6] (per os, PO) for 40 days; the dosage of the former was progressively increased from 85 to 94 mg/kg, q24h while the dosage of the latter was tapered from 8.6 to 7.8 mg/kg, q12h. Later, allopurinol was administered from day 42 to day 85 at 7.4 mg/kg, q12h, from day 85 to day 148 at 4.8 mg/kg, q12h, and then discontinued at T265. A course of metronidazole-

[5]Glucantime, Merial Italia S.p.a., Milan, Italy
[6]Allopurinolo generic, Sandoz S.p.a., Origgio, VA, Italy

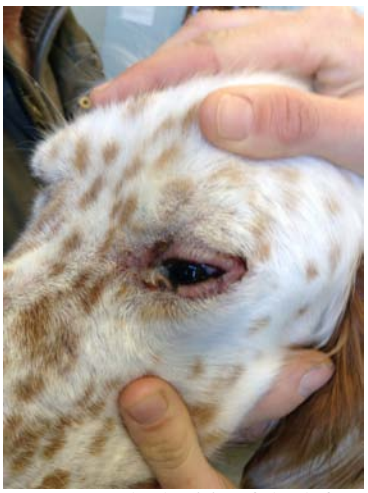

Figure 7. Blepharitis of the left eye at T3

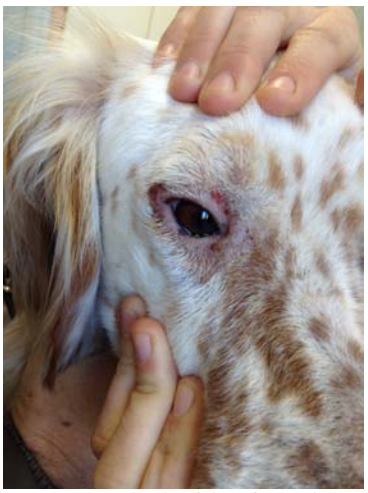

Figure 8. Blepharitis of the right eye at T3

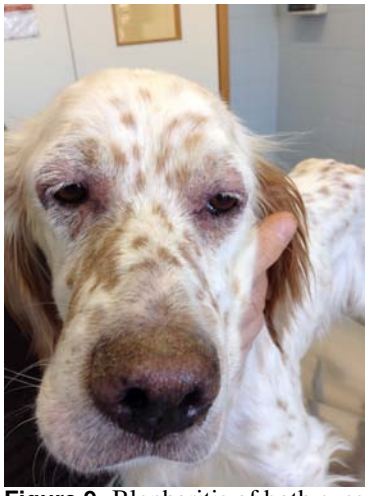

Figure 9. Blepharitis of both eyes at T10

Figure 10. Alopecia and ulcer on the tail at T10

spyramicin[7] (PO) was given up until T24 (starting at 42,860 IU/kg + 7.14 mg/kg and ending at 40,540 IU/kg + 6.75 mg/kg). Fluid therapy (saline and dextrose 5%) was provided for about 10 days, the topical treatment for the ocular complication based on eye-drops with a combination of neomycin and chloramphenicol[8] (q12h) was discontinued at T40, and the topical treatment for the skin lesion at the tail was started at T6 and continued up to T24 with a combination of iodopovidone[9] and ointment based on glycol-

[7] Stomorgyl, Merial Italia S.p.a., Milan, Italy

[8] Antibioptal, Farmila-Thea Farmaceutici S.p.a., Settimo Milanese, MI, Italy

[9] Iodopovidone, Farmacare S.r.l., San Pietro in Casale, BO, Italy

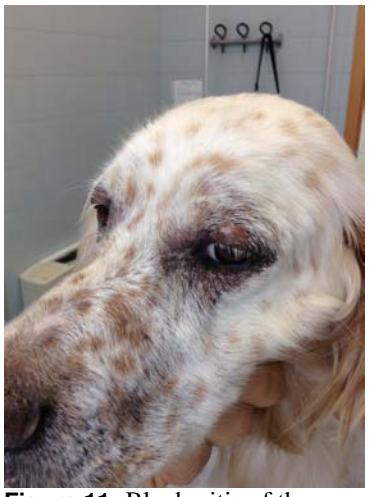

Figure 11. Blepharitis of the eyes in improvement at T24

Figure 12. Alopecia and complete recovery of the ulcer in the tail at T24

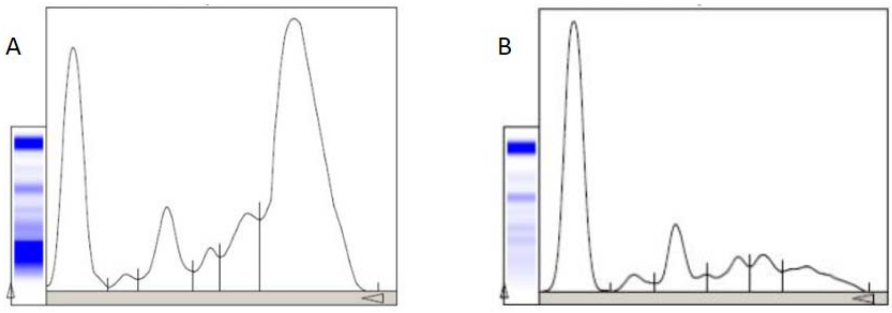

Figure 13. (A) serum protein electrophoresis showing a polyclonal peak in the gamma globulins at T0; (B) serum protein electrophoresis normalized at T148

propylene + malic acid + benzoic acid + salicylic acid[10] administered both at q12h.

Discussion and conclusion

This case describes a typical clinical presentation of leishmaniosis in a dog where breakdown of *Leishmania* vaccine or ineffective/insufficient immunity can be documented (4, 6). The case was promptly reported to the Italian Agency for Drug Surveillance in Veterinary Medicine (5). The distinction between the breakdown and ineffective/insufficient immunity could not be established as it is not common to test for ineffective/insufficient immunity as in other infectious disease. Indeed, all the serological assays available are directed at assessing the contact or exposure to *Leishmania* by a dog that could develop active infection and/or clinical disease (1).

Recently, a poster presented in an European Conference reported a case series of sixteen dogs affected by clinical signs of leishmaniosis, and previously vaccinated with Canileish® in Spain (10). The classical clinical presentation of leishmaniosis was observed in all cases and the diagnosis was based on direct visualization of *Leishmania* amastigotes by cytology, and indirectly by immunohistochemistry or molecular techniques. The majority of cases included were purebred, large and male dogs. Most of the dogs were diagnosed with clinical leishmaniosis prior to the first annual re-vaccination, and the time span between vaccination and appearance of clinical illness was 2-12 months.

The dog described in this case came from an area where leishmaniosis is known to be endemic and, therefore, at risk of developing the disease. However, the owner, a physician strictly bounded with the dog, confirmed that the use of repellent and anti-feeding agents was correctly provided to each of the three dogs living all together. All vaccination schemes were also accurately performed. Indeed, Alba was receiving the first vaccination plan in 2012 at 9 months of age, and the annual boosters in 2013 and 2014 springtime. The time elapsed from the last vaccination to the appearance of clinical signs of leishmaniosis was about 10 months. Before each annual booster the dog was screened with Speedy-Leish K® test that resulted negative.

"Alba" showed severe and typical clinical and clinico-pathological signs of *Leishmania* infection, and promptly responded to standard therapy (1, 9). All the clinical signs described resolved by T27, while the clinico-pathological signs returned to normal at different times. Indeed, while CBC results progressively returned to normal at T265, it should be noticed that at T165 the appearance of few nucleated RBCs along evidence of Howell-Jolly bodies coupled with bone marrow aspirate results showed some signs of hemopoetic dysplasia both in erythroid and myeloid series. Thereafter, the bone marrow sampling at T265 was normal. The occurrence of microcytic-hypochromic non-regenerative anemia at presentation may suggest that the clinical disease was started being present at least 3-4 months before the first admission, as RBCs half-life is 110 days. Therefore, the dog might have been infected in the summer-autumn 2014.

[10]Dermaflon, Zooetis Italia S.r.l, Rome, Italy

The biochemical profile was unrewarding from T42, and on the following monitoring. Surprisingly, the coagulation profile was always normal and particularly, the fibrinogen was never elevated despite the clinical and clinic-pathological evidence of an inflammatory process. The hallmark of the chronic disease often used for monitoring the leishmaniosis course besides the serum protein electrophoresis, was strongly modified at the initial stages but returned to normal at T148. The urinalysis followed almost the serum biochemical pattern and returned to normal by T85. The immediate diagnosis of leishmaniosis was carried out with the fine needle lymph node and the bone marrow aspirations. These two cytological assays belong to the gold standard procedures in terms of specificity and sensitivity to diagnose leishmaniosis. With appropriate therapy the sensitivity of both assays to recognize the disease is decreasing to almost zero. Of course, parasite culture could be considered but it is not suitable for rapid diagnosis, and is currently used only for research purposes. On the contrary, the qPCR can help to detect if the pathogen is still present. Serological tests, which can be time consuming if not "in house available", are the evidence of antibodies - a long lasting immunological response, and cannot be used for detecting the presence of the parasite (1, 6).

Additional drugs used to manage concurrent clinical signs were also successful in controlling all abnormalities in a short period of time. The initial administration of metronidazole-spyramicin was chosen in order to keep the dog under antibacterial coverage along with the specific therapy against leishmaniosis. The qPCR investigation, which was negative after the treatment, confirmed that the therapy instituted was beneficial.

Even if the claim of the Canileish® vaccine is expected to reduce the risk of developing an active infection, the possible immunity breakdown or ineffective/insufficient immunity and the clinical appearance of the clinico-pathological signs of disease should be kept in consideration, especially in the high risk area for *Leishmania* infection. The clinical and clinico-pathological signs of the dog reported here are not substantially different from the dogs not using the vaccine affected by leishmaniosis. The immunity against *Leishmania* should be evaluated by serological titers along with the qPCR performed either on lymph node or bone marrow aspirate samples. Furthermore, as reported by Solano-Gallego (10), the use of an accurate screening with diagnostic tests for Leishmania infection prior to vaccination in dogs living in endemic areas should be highly considered.

References

1. AAVV. (2015): Scientific Opinion on canine leishmaniosis. EFSA Journal, 13(4): 4075, 1-77.
2. Bongiorno G., Paparcone R., Foglia Manzillo V., Cuisinier A.M., Gradoni L. (2013): Vaccination with LiESPQA-21 (CaniLeish®) reduces the intensity of infection in *Phlebotomus perniciosus* fed on *Leishmania infantum* infected dogs: a preliminary xenodiagnosis study. Vet Parasitol, 197: 691–5.
3. Lemesre J.L., Holzmuller P., Goncalves R.B., Bourdoiseau G., Hugnet C., Cavaleyra M. and Papierok

G. (2007): Long-lasting protection against canine visceral leishmaniasis using the *Li*ESAp-MDP vaccine in endemic areas of France: double-blind randomised efficacy field trial. Vaccine, 25: 4223-34.

4. Paltrinieri S., Solano-Gallego L., Fondati A., Lubas G., Gradoni L., Castagnaro M., Crotti A., Maroli M., Oliva G., Roura X., Zatelli A., Zini E. (2010): Guidelines for diagnosis and clinical classification of leishmaniasis in dogs. J Am Vet Med Assoc, 236: 1184-91.
5. Report of suspected case of adverse reaction for the veterinary pharmacological surveillance (2015): http://www.salute.gov.it/portale/ministro/p4_8_0.jsp? lingua =italiano&label=servizionline&idMat=MDV&idAmb =FMV&idSrv=PSK&flag=P. Accessed April 2015.
6. Solano-Gallego L., Koutinas A., Miro G., Cardoso J., Pennisi MG., Ferrer L., Bourdeau P., Oliva G., Baneth G. (2009): Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. Vet Parasitol, 165: 1-18.
7. Martin V., Vouldoukis I., Moreno J., McGahie D., Gueguen S., Cuisinier A.M. (2014): The protective immune response produced in dogs after primary vaccination with the LiESP/QA-21 vaccine (Canileish®) remains effective against an experimental challenge one year later. Vet Res, 45: 6-21.
8. Moreno J., Vouldoukis I., Schreiber P., Martin V., McGahie D., Gueguenc S., Cuisinier A.M. (2014): Primary vaccination with the LiESP/QA-21 vaccine (CaniLeish®) produces a cell-mediated immune response which is still present 1 year later. Vet Immunol Immunop, 158: 199-207.
9. Oliva G., Nieto J., FogliaManzillo V., Cappiello S., Fiorentino E., Di Muccio T., Scalone A., Moreno J., Chicharro C., Carrillo E., Butaud T., Guegand L., Martin V., Cuisinier A.M., McGahie D., Gueguen S., Canavate C., Gradoni L. (2014): A Randomised, double-blind, controlled efficacy trial of the LiESP/QA-21 vaccine in naive dogs exposed to two Leishmaniainfantum transmission seasons. PLOS Negl. Trop. Dis, 1-10.
10. Solano-Gallego L., Tabar M.D., Homo F., Ordeix L. (2016): A descriptive study of clinical canine leishmaniosis in dogs vaccinated with Canileish. Proc. Eur Coll Vet Internal Med - Comp Anim, Goteborg, SV, Sept 8-10, 2016, 368

Supplement

Table 1. Clinical monitoring of Alba

	T0	T3	T10	T24
Weight kg	17.5	17.7	18.5	19.1
BCS	2/9	2/9	3/9	3/9
Temperature °C	39.6	39.2	38.9	38.3
Pulse ppm	104	120	110	80
Breath bpm	36	32	30	15
Mucous membranes	Light pink	Pale pink	Pink	Pink
Eyes	sero-mucous discharge and photofobia	sero-mucous discharge and mild photofobia	Sero-mucous discharge and blepharitis	Mild blepharitis
Lymphatic	lymph node enlargement swollen and bloody at punction	lymph node enlargement more harden	lymph node enlargement more harden	Mild lymph node enlargement more harden
Skeleton	forelimb lameness from bilateral carpal swollen (mostly in the right side)	Right carpal swollen	Right carpal swollen (reduced)	Absent
Skin	NtR	NtR	Generalized furfuraceous desquamation and alopecia and large ulcer on the tail	Only alopecia on the tail

Notes: at T42, T85, T148 and T265 clinical signs were unremarkable and Alba gained her body weight of 21 kg with a normal BCS (Body Condition Score) of 4/9
NtR -nothing to report

Table 2. CBC monitoring of Alba

Analyte	Reference Ranges	Units	T0	T6	T10	T24
RBC	5.66-8.87	M/μL	4.37	4.48	4.53	4.66
HCT	37.3-61.7	%	23.9	25	26	27.8
HGB	13.1-20.5	g/dL	8.5	8.8	9	9.7
MCV	61.6-73.5	fL	54.7	55.8	57.4	59.7
MCH	21.2-26.9	pg	19.5	19.6	19.9	20.8
MCHC	32.0-37.9	g/dL	35.6	35.2	34.6	34.9
RDW	13.6-21.7	%	17.4	17.5	18.9	21.1
Retics	10.0-110.0	K/μL	17	30.9	62.5	72.2
Estimate Retics	<60.0	K/μL	21.9	22.4	68	93.2
WBC	5.05-16.76	K/μL	5.23	4.68	8.04	6.96
NEU	3.7-11,9	K/μL	3.03	2.62	3.84	4.45
BAND	0.0-0.3	K/μL	0	0	0	0
LYM	0.7-5.1	K/μL	1.46	1.59	3.52	1.81
MONO	0.2-1.7	K/μL	0.63	0.37	0.64	0.28
EOS	0.1-1.35	K/μL	0.1	0.09	0	0.42
BASO	0.0-0.1	K/μL	0	0	0	0
PLT	148-484	K/μL	132	123	338	363
MPV	8.7-13.2	fL	10.8	NA	9.9	9.6
Estimate PLT	Adequate		Reduced	Reduced	Adequate	Adequate
RBC morphology			Anysocytosis +, poikylocytosis +, target cells +	Anysocytosis +	NRBC #2, polychromasia+, Anysocytosis ++	polychromasia +, Anysocytosis ++
WBC morphology			Reactive lymphocyte ++	Reactive lymphocyte ++	Reactive lymphocyte ++	Reactive lymphocyte ++
TPP refractometer	5.8-7.8	g/dL	10	10	10.4	10.5

Notes: ND- not determined

Table 2. CBC monitoring of Alba (Continued)

Analyte	Reference Ranges	Units	T42	T85	T148	T265
RBC	5.66-8.87	M/μL	5.45	5.49	5.78	5.64
HCT	37.3-61.7	%	33.4	33.9	37.8	35.5
HGB	13.1-20.5	g/dL	11.5	11.8	13.2	12.6
MCV	61.6-73.5	fL	61.3	61.7	65.4	66.5
MCH	21.2-26.9	pg	21.1	21.5	22.8	23.4
MCHC	32.0-37.9	g/dL	34.4	34.8	34.9	35.2
RDW	13.6-21.7	%	20.5	19.5	15.3	15.3
Retics	10.0-110.0	K/μL	40.9	34.6	79.2	40.1
Estimate Retics	<60.0	K/μL	ND	ND	ND	ND
WBC	5.05-16.76	K/μL	6.97	8.43	8.21	8.91
NEU	3.7-11,9	K/μL	3.15	3.96	4.06	5.31
BAND	0.0-0.3	K/μL	0.07	0	0	0
LYM	0.7-5.1	K/μL	3.36	2.53	3.77	2.68
MONO	0.2-1.7	K/μL	0.14	1.18	0.2	0.71
EOS	0.1-1.35	K/μL	0.28	0.67	0.16	0.2
BASO	0.0-0.1	K/μL	0	0.08	0	0
PLT	148-484	K/μL	410	306	278	315
MPV	8.7-13.2	fL	9.4	9.6	9.5	9.1
Estimate PLT	Adequate		Adeguate	Adequate	Adequate	Adequate
RBC morphology			Anysocytosis +	Anysocytosis +, Keratocyte +/-	NRBC #1, polychromasia +, Anysocytosis +, Howell-Jolly bodies +/-	Anysocytosis +,
WBC morphology			Reactive lymphocyte +			
TPP refractometer	5.8-7.8	g/dL	9.4	7	6.1	6.9

Notes: ND- not determined

Table 3. Serum biochemical profile monitoring of Alba

Analyte	Reference ranges	Units	T0	T24	T42	T85	T148	T265
Total calcium	8.7-11.8	mg/dL	10.5	10.7	10.2	9.6	9.8	10.6
Phosphates	2.5-5.0	mg/dL	4.6	2.6	4.7	4	3.5	3.5
Iron	80-190	μg/dL	95	321	NT	NT	116	200
Total proteins	5.8-7.8	g/dL	11.5	12.3	9.4	7.4	6	6
Albumin	2.6-4.1	g/dL	1.9	2.5	2.6	2.9	3.2	3.1
Globulins	2.5-4.5	g/dL	9.7	9.8	6.8	4.5	2.8	2.9
A/G ratio	0.5-1.5		0,20	0.25	0.38	0.64	1.14	1.06
Fructosamine	170-430	μmol/L	198	252	NT	NT	325	329
C-reactive protein	0.0-0.30	mg/dL	2.3	0.2	0.2	NT	0.3	0.3
Urea	15-55	mg/dL	17	20	33	20	34	14
Creatinine	0.6-1.5	mg/dL	1	1	1	0.9	1.1	0.8
Total bilirubin	0.07-0.30	mg/dL	0.11	0.07	NT	0.2	0.2	0.07
Cholesterol	120-280	mg/dL	206	212	263	280	218	208
Tryglicerides	25-90	mg/dL	75	75	NT	NT	52	69
Glucose	80-125	mg/dL	104	101	117	114	105	120
Alkaline phosphatase (ALP)	45-250	U/L	180	120	105	104	110	302
Gammaglutamyl transferase (GGT)	2.0-11.0	U/L	4	2	2	1	6	3
Aspartateaminotransferase (AST)	15-40	U/L	75	27	NT	NT	35	21
Alanineaminotransferase (ALT)	20-70	U/L	22	22	25	69	174	44
Creatinphosphokinase (CK)	40-185	U/L	177	184	NT	NT	69	56
Lactatedehydrogenase (LDH)	20-160	U/L	338	166	NT	NT	57	91
Amylase	400-1500	U/L	1228	1362	NT	486	839	563
Sodium	146-156	mEq/L	152	158	157	153	146	156
Potassium	3.9-5.5	mEq/L	4	4	4.5	4	3.6	4.2
Chloride	109-122	mEq/L	115	117	118	117	116	118
Bicarbonate	21-31	mEq/L	24	26	20.5	NT	28	29

Notes: NT- not tested

Table 4. Coagulation profile monitoring of Alba

Analyte	Reference Ranges	Units	T0	T24	T148	T265
Prothrombin time	5.4-8.1	Seconds	9	7.1	7.1	7.3
Partial thromboplastin time	10.7-17.5	Seconds	17.5	15.9	14	11.7
Fibrinogen	125-335	mg/dL	275	241	177	300

Table 5. Serum protein electrophoresis monitoring of Alba

Analyte	Reference ranges	Units	T0	T24	T85	T148	T265
Albumin	47.5-58.5	%	22.2	20	37.5	52.7	51.7
Alpha-1-globulin	2.6-4.4	%	1.4	1.9	5.7	3.8	3.6
Alpha-2-globulin	10.7-18.0	%	8.7	6.9	15.8	14.3	15.7
Beta-1-globulin	5.9-13.3	%	3.3	6.7	10.9	8.7	11.1
Beta-2-globulin	7.2-14.4	%	9.2	7.3	10.2	8.5	9.5
Gamma globulin	6.6-11.6	%	55.2	57.2	19.9	12	8.4
A/G	0.5-1.5		0.29	0.26	0.6	1.11	1.07
Total protein	5.8-7.8	g/dL	11.5	12.3	8.1	6	6

Table 6. Urinalysis monitoring of Alba

Analyte	Reference ranges	Units	T0	T148
Method of collection			Cystocentesis	Cystocentesis
Color	Yellow	NA	Yellow-green	Yellow
Aspect	Clear	NA	Cloudy ++	Cloudy +
Specific gravity	1018-1045	NA	1035	1030
pH	5-7	NA	8	6
Protein	<40	mg/dL	30	Absent
Glucose	absent	mg/dL	Absent	Absent
Ketones	Absent	mg/dL	Absent	Absent
Urobilinogen	Absent	mg/dL	Absent	Absent
Bilirubin	Absent	mg/dL	Absent	Absent
Blood	Absent	RBCs/mcL	50	Absent
Leukocytes	Absent	WBC/mcL	25	Absent
UP/C ratio	0.1-0.5	NA	0.64	0.15
Cellularity	Rare	NA	Moderate	Moderate
RBC	1-5	HPF	++	Absent
WBC	1-5	HPF	-/+	-/+
Epitelial cells	1-5	HPF	+	-/+
Other		NA	Lipid droplets	

Notes: NA – not applicable

Table 7. Lymph node evaluation of Alba

	At T0	At T265
Smear quality	Good but some hemodilution	Very good
General observation	Polymorphic cell pattern	Polymorphic cell pattern
Lymphoid cells	Small lymphocytes prevalent (ca. 80%), lymphoblasts or immunoblasts (ca. 10%), plasmacells (ca. 10%)	Small lymphocytes prevalent, few immunoblasts and plasmacells
Other cells	A great number of macrophages some with *Leishmania* spp	Rare mastocytes and macrophages
Mitosis	Some occurring but typical	Not observed
Conclusion	Lympho-plasmacellular hyperplasia compatible with *Leishmania* infection	Normal lymphonode with a mild increase of plasmacells

Table 8. Bone marrow monitoring (smear evaluation and PCR) of Alba

Analyte	T0	T148
Collection site	Iliac crest	Sternum
Cellularity	Very good	Good
Megakariocytes	Occurring in all maturation forms	Occurring in all maturation forms
Myeloid series	Proliferative, maturative, and storage cells well represented	Proliferative and maturative cells well represented, less cells in the storage pool
Erythroid series	Proliferative and maturative cells well represented	Proliferative and maturative cells well represented
Other cells	Rare lymphocyte and plasmacells – macrophages with *Leishmania* spp.	Rare plasmacells
Mitosis	Occurring in myeloid and erythroid series	Occurring in myeloid and erythroid series – some atypical in myeloid
Conclusions	Megakariocyte, myeloid and erythroid series normal for number and morphology. Mild hyperplasia of myeloid in comparison to erythroid series. Macrophages with *Leishmania* spp.	Megakariocyte series normal for number and morphology. Myeloid normal for number but mild sign of dysplasia. Erythroid series normal for morphology mild prevalence to myeloid.
Anaplasma pahogocytophilum/platys (PCR+Hybridization Hybprobes)	Negative	NT
Babesia spp (conventional PCR)	Negative	NT
Bartonella spp (PCR+SYBR Green®)	Negative	NT
Ehrlichia canis (PCR+Hybridization Hybprobes)	Negative	NT
Hepatozoon spp (conventional PCR)	Negative	NT
Leishmania infantum (PCR+Hybridization Hybprobes)	Positive (1.212.000.000 copies)	Negative (limit 100 copies of kinetoplast)
Rickettsia spp (PCR+Hybridization Hybprobes)	Negative	NT

Notes: NT- not tested

Table 9. Treatment monitoring of Alba

T0 (kg. 17.5)	T3 (kg. 17.7)	T10 (kg. 18.5)
Meglumine antimonate = 85.7 mg/kg SC	Meglumine antimonate = 84.7 mg/kg SC	Meglumine antimonate = 97.3 mg/kg SC
Allopurinol = 8,6 mg/kg bid OS	Allopurinol = 8.5 mg/kg bid OS	Allopurinol = 8.1 mg/kg bid OS
Spiramycin = 42,860 UI/kg bid OS	Spiramycin = 42,370 UI/kg bid OS	Spiramycin = 40,540 UI/kg bid OS
Metronidazole = 7.14 mg/kg bid OS	Metronidazole = 7.06 mg/kg bid OS	Metronidazole = 6.75 mg/kg bid OS
Glucose 5% = 14.3 ml/kg bid EV	Glucose 5% = 14.1 ml/kg bid SC	interrupted
Multi-vitamins vials SC	Multi-vitamins vials SC	Multi-vitamins vials SC
Artificial lacrimal drops TID topical	Artificial lacrimal drops TID	Artificial lacrimal drops TID
Chloramphenicol/Neomicin eye drops TID topical	Chloramphenicol/ Neomicin eye drops TID	Chloramphenicol/ Neomicin eye drops TID
	Topical use on tail skin of iodopovidone BID	Suspended when the ulcer was healed
	Topical use of combined drug: Glycol-propilene + malic acid + benzoic acid + salicylic acid	Topical use of combined drug: Glycol-propilene + malic acid + benzoic acid + salicylic acid

Table 9. Treatment monitoring of Alba (Continued)

T24 (kg. 19.1)	T42 (kg. 20.3)	T85 (kg. 20.8)	T148 (kg. 21.3)
Meglumine antimonate = 94.2 mg/kg SC	Interrupted at T40		
Allopurinol = 7.8 mg/kg bid OS	Allopurinol = 7.4 mg/kg bid OS	Allopurinol = 4.8 mg/kg bid OS	interrupted
interrupted			
interrupted			
Multi-vitamins vials SC	Multi-vitamins tablet OS	Multi-vitamins tablet OS	interrupted
Artificial lacrimal drops TID	interrupted		
interrupted			
interrupted			
interrupted			

Tipična lajšmanioza psa redovno vakcinisanog sa Canileish® vakcinom

Sažetak

Uvod

Vakcina protiv lajšmanioze pasa (Canileish®) napravljena od LiESAp i 54-kDa proteina estrahovanih iz *Leishmania infantum*, a uz dodatak muramil-peptida (MDP) kao adjuvantnog sredstva, koristi se u nekoliko evropskih država.

Razvijena je da stimuliše ćelijski imunitet kod pasa serološki negativnih na lajšmaniozu, kako bi se smanjio rizik od razvoja infekcije i kliničkih manifestacija bolesti. U ovom članku je opisan slučaj psa sa teškim oblikom lajšmanioze, koji je redovno vakcinisan sa (Canileish®) vakcinom i tretiran repelentima i drugim sredstavima protiv ektoprazita (vektora).

Materijal i metode

"Alba", engleski seter, intaktna ženka stara tri godine dovedena je (mart 2015.) sa simptomima dizoreksije, gubitka tjelesne mase, sa povišenom temperaturom i slabošću prednjih nogu. "Alba" je uz prevenciju ekto i endoparazitoza redovno vakcinisana protiv najčešćih bolesti pasa, te Canileish® vakcinom protiv lajšmanioze.

Fizikalnim pregledom (T0[11]) su zabilježeni: indeks tjelesne kondicije 2/9, temperatura 39.6 °C, umjereno povećanje perifernih limfnih čvorova, seropurulentni iscjedak iz oka sa fotofobijom i otečen i bolan desni karpalni zglob.

Urađena je kompletna krvna slika (CBC), biohemijski profil, koagulacijski profil, elektroforeza serumskih proteina, serološko testiranje na *L. infantum*, analiza urina uključuju i UP/C omjer, punkcija preskapularnih i poplitealnih limfnih čvorova, punkcija koštane srži za citologiju i qPCR na L. infantum.

Rezultati

Različiti klinički simptomi su se povukli sukcesivno: slabost prednjih ekstremiteta - T3, temperatura i fotofobija - T10, seropurulentni iscjedak iz oka - T15, blefaritis, limfadenopatija i velike kožne promjene repa - T24, tjelesna težina T42.

Kompletna krvna slika (T0) je pokazala umjerenu mikrocitnu neregenerativnu anemiju, blagu neutropeniju i trombocitopeniju (normalni na T265). Biohemijski profil (T0) je potvrdio tešku hiperproteinemiju (normalne vrijednosti na T85), hipoalbuminemiju (normalno na T42), hiperglobulinemiju, nizak albuminsko/globulinski omjer (oboje normalni na T85), i povišen C-reaktivni protein (normalan na T24). Elektroforeza serumskih proteina (T0) je pokazala hipoalbuminemiju i poliklonalnu hipergamaglobulinemiju (normalna na T148).

Analizom urina (T0) ustanovljena je hematurija (normalno na T6) i blagi porast omjera proteina i kreatinina u urinu (normalni na T85). U aspiratima limfnih čvorova i koštane srži (T0) utvrđene su amastigote lajšmanije.

Uzorci limfnih čvorova i koštane srži bili su negativni qPCR tehnikom na *Babesia* spp, *Bartonella* spp, *Ehrlichia canis*, *Hepatozoon* spp, i *Rickettsia* spp, ali pozitivni na *L. infantum* (negativni na T148 i T265).

IFAT metodom (T0) je ustanovljen izrazito visok titar antitijela (1:5120), koji se smanjio na 1:320 (T148) i 1:160 (T265). Speed-Leish K® test je cijelo vrijeme bio pozitivan.

"Alba" je tretirana sa meglumin-antimonatom (SC) i alopurinolom (PO) u trajanju od 40 dana. Alopurinol je isključen na T265. Metronidazol-spiramicin je apliciran perooralno do T24. Terapija tečnostima (fiziološki rastvor NaCl i 5% glukoze) aplicirana je deset dana, topikalna terapija repa je prekinuta na T24, a oka na T40.

Interpretacija i zaključak

Ovaj slučaj opisuje tipičnu kliničku i kliničko-patološku sliku lajšmanioze nakon neuspjeha vakcine ili neefikasnog/nedovoljnog imuniteta.

Vlasnik psa je potvrdio korištenje repelenta i drugih sredstava protiv vektora te redovnu vakcinaciju. Posljednja doza Canileish® je aplicirana oko 10 mjeseci prije izbijanja bolesti.

"Alba" je pokazala brz odgovor na standardnu terapiju. Svi opisani klinički znaci su se povukli do T27, dok su se kliničko-patološki znaci vraćali u normalne granice sukcesivno.

Kompletna krvna slika i citološki pregled koštane srži su se progresivno vratili u normalne granice na T265. S obzirom na pojavu mikrocitne hipohromne neregenerativne anemije na T0, moguće da je pas zaražen u ljeto/jesen 2014. godine. Biohemijski profil i analiza urina nisu bili signifikantni od T42, odnosno T85. Elektroforeza serumskih proteina je znatno odstupala od normalnih vrijednosti na T0, ali se normalizirala na T148. Neposredna dijagnoza lajšmanioze je postavljena ciljanom punkcijom limfnog čvora i biopsijom koštane srži, za koje je poznato da imaju visoku specifičnost i osjetljivost. Senzitivnost oba testa opada pod djelovanjem terapije tako da qPCR može pomoći u otkrivanju patogena, i utvrđivanju uspješnosti terapije. Serološki dokaz antitijela se ne može koristiti kao potvrda prisustva parazita u organizmu jer se antitijela jako dugo zadržavaju.

Opisani slučaj potvrđuje da i pored vakcinacije postoji mogućnost pojave kliničkih i drugih znakove lajšmanioze, što treba imati u vidu posebno u područjima koja su visokorizična za pojavu lajšmanioze.

[11] T: vrijeme u danima od dana prijema (T0) pacijenta