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# Effective or flawed vaccine-related immune surveillance in a dog vaccinated with LiESP/QA-21: cutaneous leishmaniosis? An account of a specific incident

**ABSTRACT**: Because of its association with a broad variety of human and animal illnesses, the intracellular protozoan parasite leishmania is a major public health concern in many Mediterranean basin nations. Vaccination of dogs is still the most effective method of controlling the spread of canine diseases in regions where the illness is mostly transmitted to people via zoonotic means. This case study details the presentation of a four-year-old canine with a nodular skin lesion to a private clinic after having received a canine leishmaniosis vaccine. Histopathological analysis of the excised skin lesion, in addition to normal biochemical and haematological tests, showed that Leishmania amastigotes were present. It is suggested that the protozoa may be present in a vaccinated dog's skin lesion.

Keywords: Vaccine, amastigote, Leishmania infantum, cutaneous noduleCase description

This report describes a case of an atypical form of canine leishmaniosis observed in a 4-year-old male Boxer dog with a history of regular vaccina- tion course against Leishmania infantum (LiESP/QA-21, CaniLeish<sup>®</sup>, Virbac France). The complete primary vaccination course of three injections started when the dog was 18 months old, subse- quently continuing with an annual booster vacci- nation. The owner brought the animal to a private vet- erinary clinic (April 2015) for the appearance of a cutaneous nodule on its head (the parietal area) that had started to grow over the preceding three months. The booster vaccine dose had been ad- ministered seven months previously. Physical examination revealed that the dog was in good health condition, alert, hydrated, afebrile and without other systemic signs, including local or peripheral lymphadenopathy. The nodular skin lesion had the appearance of a "cabbage" excrescence with a "chickpea" diameter (about 1 cm); it was not ulcerated, and seemed to be the sole manifestation of the skin disease.

Initially, the differential diagnosis of the lesion included skin tumours (histiocytomas, mast cell tumours, cutaneous lymphoma), infectious and sterile granulomas, eosinophilic granulomas, cuta- neous cysts (Ferrer et al. 1990; Koutinas et al. 1992), cutaneous abscesses, subcutaneous helmintho- sis caused by Dirofilaria repens and mycetomas (Blavier et al. 2001). Under an appropriate sedation, the nodular lesion was removed, and histopatho- logical examination revealed follicular keratosis and a severe and chronic inflammatory infiltrate. The latter, localised particularly near the skin annexes, was composed of many histiocytes and plasma cells, a moderate number of mature lym- phocytes, a few eosinophils and rare neutrophils, and detectable leishmanial amastigote  $(2-3 \mu m \text{ in diameter})$  forms in the macrophages (Figure 1).

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Figure 1. Skin, within the cytoplasm of a macrophage and between inflammatory cells numerous  $2-4 \mu m$ , ovoid, protozoan amastigotes are visible (× 100 magnification)

After histopathological diagnosis, serum bio- chemical analysis was performed and the total titres of anti-*Leishmania* antibodies were determined us- ing an indirect immunofluorescence antibody test (IFAT) (MegaFLUO<sup>®</sup> LEISH; Megacor Diagnostik Gmbh).

Table 1. Serum haematological profile evaluated in a 4-year-old male dog at the first clinical examination  $-1^{st}$  blood sampling (BS), and after eight months  $-2^{nd}$  BS

Parameter	1 <sup>st</sup> BS	2nd BS	Range
RBC (10 <sup>6</sup> /µl)	8.16	9.18	5.8-8.8
WBC (10 <sup>3</sup> /µl)	10.9	10.8	5.5-15.5
PLT (10 <sup>3</sup> /µl)	199	163	150-500
Urea (mmol/l)	14.81	16	6.43–19.6
Creatinine (µmol/l)	83.1	98.1	70.7-123.8
Glucose (mmol/l)	6.6	5.01	3.3-6.05
Fructosamine (µmol/l)	338	349	160–350
AST (IU/l)	29	37	15-40
ALT (IU/l)	72	83	15–55
Protein (g/l)	66.7	72.2	56–78
Albumin (g/l)	36.1	39.8	26.3-45.3
α1-globulin (g/l)	3.3	3.6	1.9–3.4
α2-globulin (g/l)	9.3	10.7	9–16.1
β1-globulin (g/l)	4.7	4.7	2.7-10.2
$\beta$ 2-globulin (g/l)	6.1	6.3	3.4-8.7
γ-globulin (g/l)	7.2	7.1	3–7.8
Albumin/globulin (ratio)	1.17	1.22	0.6-1.41

PLT = platelets, RBC = red blood cells, WBC = white blood cellsThe IFAT provided a negative result (titre of 1 : 40; positive test result is  $\geq$  50) and the complete blood count (by ADVIA 2120 V automated counter) revealed a normal haematological profile (Table 1; first blood sample = 1<sup>st</sup> BS). The serum biochemical indicators (by Architect c8000 Clinical Chemistry Analyzer, Abbott Diagnostics, USA), reported in Table 1 (1<sup>st</sup> BS), revealed a normal pherogram pro- file and serum protein levels as well as a moderate increase in alanine aminotransferase. For this rea- son, the dog was not treated against *Leishmania*, and eight months after the diagnosis (December 2015), the dog was still in good body condition at the



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physical examination, with blood and serum parameters within the physiological range (Table 1; second blood sample =  $2^{nd}$  BS). The IFAT again provided a negative result (titre of 1 : 40).

## DISCUSSION AND CONCLUSIONS

*Leishmania* is an intracellular protozoan para- site that is endemic, widespread and represents a public health problem in most countries of the Mediterranean basin as it is implicated in a wide spectrum of diseases both in humans and animals. Leishmaniosis currently threatens 350 million people in 98 countries, with 1.2 million new cases per year (Handler et al. 2015; Kahime et al. 2015). In regions where *L. infantum* is endemic, canine seropreva- lence ranges from 5% to 37% (Alvar et al. 2004), with dogs also playing an important epidemiological role as a reservoir of infection for humans (Viegas et al. 2012). After inoculation by the sand fly, the parasites disseminate towards the lymph nodes, bone marrow and viscera, such as the spleen and liver, where they replicate, causing disease. Depending on the ability of the host's immune response to control parasite replication, the infection in dogs could range from subclinical/asymptomatic to fully developed disease (Alexandre-Pires et al. 2010). Skin lesions caused by *Leishmania* have been well-described but the nodular form of canine leishmaniosis is much less frequent (Blavier et al. 2001).

According to some authors (Ferrer et al. 1988; Vidor et al. 1991; Amara et al. 2000), the nodular form of leishmaniosis is more commonly diagnosed in boxers that, together with the Shepherd dogs, seem to be the breeds more predisposed to overt disease (Paltrinieri et al. 2010). Two cases of atypi- cal nodular forms, one in the interdigital space,

and the other in the right axilla were reported by Ferrer et al. (1990) in dogs that were not vaccinated. These nodules, as the one described here, were not ulcerated. Other reports of atypical nodular lesions caused by *Leishmania* have been reported on a dog tongue (Blavier et al. 2001; Viegas et al. 2012). All these animals, however, also showed other findings attributable to leishmaniosis (i.e. positive serology or the presence of *Leishmania* in the bone mar- row). Although in our presented case it was not possible to exclude dissemination to the bone mar- row through cytological or PCR tests (the owner did not consent to these further investigations), a seroconversion was neither observed at the first visit nor eight months later.

Although the nodular lesion observed in the pre- sent boxer dog could be attributable to *Leishmania* as the causative agent, it was not an atypical clinical manifestation of disseminated infection. Rather, it stemmed from a strong non-suppurative inflam- matory reaction to the entrance of the *Leishmania* protozoa at the bite site of sand flies, in an immune- competent animal.

The vaccine that is administered to dogs acts by developing long-lasting cell-mediated immune responses against *L. infantum*, specifically with a domi- nant  $CD4^+$  Th1 influence in an overall mixed cellular response. Among all the activated inflammatory cells, macrophages show a strong ability to reduce the levels of intracellular parasites through the induction of in- ducible nitric oxide synthase and production of nitric oxide derivatives (Moreno et al. 2014). This vaccine, recently licensed in Europe, displayed 68% to 92% efficacy in protecting animals against the appearance of clinical signs, under field conditions (Otranto and Dantas-Torres 2013; Oliva et 2014).

In this case, even if the vaccination was not able to

prevent the entrance of the protozoa into the dog, it seems it allowed the animal to effectively control the infection and remain healthy. This hypothesis is supported by the absence of any clinical, serological and haematological symptoms other than the observed nodular lesion, both at the moment of the physical examination and eight months later. Based on its histopathological features, the skin lesion could represent the result of a local reaction mediated by the cellular immune response against the attempt of protozoa to enter and disseminate in the dog.

Vaccination remains the best control strategy to counteract the progression of active infection



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forcanine disease in areas of the world where trans- mission to humans is primarily zoonotic (Dye 1996; Alvar et al. 2004). This field case further supports that hypothesis. Furthermore, if the immune system directly controls the parasite's replication making the infected dogs less infectious to same sand flies (Bongiorno et al. 2013), this in turn could further reduce the number of dogs infected by sand flies (Moreno et al. 2014).

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