



## Research paper

## Comparison of efficacy and safety of preventive measures used against canine leishmaniasis in southern European countries: Longitudinal retrospective study in 1647 client-owned dogs (2012–2016)

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## ABSTRACT

The best preventive strategy for canine leishmaniasis is, to date, unknown. In the last few years, new preventive measures have become available in Europe, including vaccination against leishmaniasis and the use of domperidone. The objective of the present study was to evaluate the efficacy and safety of the different preventive measures available against leishmaniasis in client-owned dogs.

A database search of medical records was carried out in 52 private veterinary practices located in endemic areas of canine leishmaniasis in Spain, Italy and Portugal. Healthy seronegative dogs were included in the study. Serology was repeated at least 6 months later, and was used to retrospectively classify dogs into healthy, infected or sick.

A total of 1647 dogs were included in the study. The use of preventive measures in this population was widespread. The single most utilized measure was repellents alone in 45.7% of dogs, followed by the combination of repellents and vaccination in 23.0%, repellents and domperidone in 11.3%, vaccination alone in 4.2%, vaccination and domperidone in 2.7%, domperidone alone in 2.3%, and the combination of the three measures in 0.2% of dogs. No preventive measure was applied in 10.7% dogs.

The incidence of clinical leishmaniasis in the group with no preventive treatment applied was 12.5%. In the groups where prevention was applied, the reported incidence was the following: 10.1% for the vaccination only group, 4.5% for repellents only group, 4.0% for repellents + vaccination group, and 0.5% for repellents + domperidone group. No dogs in the groups of domperidone, vaccination + domperidone, and combination of the three measures developed clinical leishmaniasis. All preventive measures resulted in a significantly lower incidence of leishmaniasis compared to not applying any measure, except for vaccination alone. The majority of preventive strategies used, with exception of vaccination alone, decreased the incidence of leishmaniasis significantly.

Adverse events, mild and self-limiting in most of the cases, were reported in 5.2% of dogs and were significantly more common in dogs following vaccination.

In conclusion, this is the first large-scale field study investigating the efficacy and safety of the preventive measures used routinely against leishmaniasis in client-owned dogs. Most preventive strategies used, with exception of vaccination alone, had some benefit over not applying any preventive. In this field study, the use of repellents showed a good degree of protection in dogs living in endemic areas of canine leishmaniasis. Although lower numbers of dogs are included, the use of domperidone appeared to provide additional protection. The role of vaccination and its combination with other preventive strategies needs further study.

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## 1. Introduction

Canine leishmaniasis is a vector-borne zoonotic disease endemic in southern Europe caused by the protozoan *Leishmania infantum* (Alvar et al., 2012). Over the last decades, many efforts have been dedicated to developing and evaluating new tools to prevent canine leishmaniasis (Maroli et al., 2010; Miró et al., 2017). Previously, these efforts were focused on the development of synthetic pyrethroids to protect dogs from sandfly vectors, which showed an efficacy ranging from 50 to 100% (Maroli et al., 2010; Miró et al., 2017). More recently, the focus has shifted towards preventing clinical disease development using immunoprophylaxis, resulting in the introduction of vaccines, drugs, as well as nutraceuticals (Reguera et al., 2016). Following this direction, in Europe, a LiESP/QA-21 vaccine (CaniLeish®, Virbac) against leishmaniasis was first registered for use in dogs in 2011. In 2016, another canine vaccine based on the recombinant protein Q (Letifend®, Leti) was launched (Fernández Cotrina et al., 2018; Oliva et al., 2014; Solano-Gallego et al., 2017). Furthermore, domperidone (Leisguard®, Ecuphar), a dopamine D2 receptor antagonist with an immunomodulatory effect through the increase in prolactin blood levels, has also become available in an attempt to prevent and control leishmaniasis in dogs (Sabaté et al., 2014). Finally, oral dietary nucleotides and active hexose correlated compounds (Impromune®, Bioiberica) have been recently reported to exert a preventive effect and decrease disease progression in clinically healthy *Leishmania*-infected dogs (Segarra et al., 2018).

Although many preventive measures are currently available, the best combination strategy to prevent leishmaniasis in dogs remains to be known. Efficacy studies have focused on one particular product under controlled conditions. Therefore, field studies on the benefit of combination measures on the field are lacking (Otranto and Dantas-Torres, 2013). The main objective of the study was to compare the efficacy and safety of different preventive measures individually, and in combination, against leishmaniasis in client-owned dogs.

## 2. Material and methods

### 2.1. Study population

A database search of medical records was carried out in 52 private veterinary practices located in endemic areas of canine leishmaniasis in Spain, Italy and Portugal between January 2012 and May 2016. To be included in the study, dogs had to fulfill the following criteria: 1) to be clinically healthy and *Leishmania* seronegative, 2) to have repeated serological results available at least 6 months after the initial testing, 3) to include one transmission season (comprising the period between June and September) within the follow-up period, and 4) to have consistently received the same preventive measures throughout the follow-up period following the manufacturer's recommendations.

Veterinarians were asked to fill out an electronic questionnaire with the information of each dog's medical record. Data collected included signalment (gender, age, weight, breed), information on initial and final

serology tests (date, type and result), risk of exposure ("high risk" was defined as dogs living outdoors or dogs living indoors but spending much time outdoors in hours of high activity of the vector; "low risk" was defined as dogs living indoors and not spending much time outdoors in hours of high activity of the vector). In addition, preventive measures used (type/s, route and dose of administration) and adverse events observed attributed to the preventive measures were reported. Finally, presence, clinical signs and/or clinicopathologic abnormalities associated with leishmaniasis, as well as, results of parasitological tests for the diagnosis of *Leishmania* infection, if available, were reported.

### 2.2. Preventive measures applied

Dogs were classified in the following groups according to the preventive measures applied during the study period: no measure applied (NONE group), use of repellents only (REP group), use of vaccination only (VACC group), use of domperidone only (DOMP group), or combinations of the above [repellents and vaccination (R + V), repellents and domperidone (R + D), vaccination and domperidone (V + D), and combination of the three measures (R + V + D)].

### 2.3. Classification of clinical leishmaniasis

Dogs were classified based on their clinical leishmaniasis status in 3 categories: healthy, infected or sick. The guidelines published by the Canine Leishmaniasis Working Group (Paltrinieri et al., 2010; Roura et al., 2013) were adapted by three European veterinary internal medicine board certified authors (MF, LT, XR) as a guide for classification. Classification criteria used in the present study can be found in Table 1.

### 2.4. Adverse events

The occurrence and type of suspected adverse events were recorded in the questionnaire. When more than one preventive measure was applied, the veterinarians were also asked to identify the preventive measure most likely associated with that event.

### 2.5. Statistical analysis

All statistical analyses were performed using commercially available software<sup>1</sup>. Significance was set at  $p < 0.05$ . Descriptive statistics are presented to define population. Continuous variables are expressed as mean  $\pm$  standard deviation.

Homogeneity of the groups organized by the preventive measures used was assessed for each of the following variables: gender, age, breed, weight, and duration of exposure (defined as the interval between the first and last serology test) and risk of exposure. For the categorical variables (gender, breed and risk of exposure), the chi-square test was used for global comparison. In case significant differences were found for a given variable, the Fisher test was used for comparison of every pair of preventive measures groups, with the  $p$ -

**Table 1**

Classification criteria used in this study based on Canine Leishmaniasis Working Group staging (Paltrinieri et al., 2010).

Category	Classification criteria
Healthy	- Dogs with no clinical signs or clinicopathologic abnormalities with negative serology, and also negative parasitological tests if performed
Infected	- Dogs with no clinical signs or clinicopathologic abnormalities and vaccinated against leishmaniasis with serology $\leq 1/160$
	- Dogs with no clinical signs or clinicopathologic abnormalities (independent of parasitological results) with positive qualitative serology or quantitative serology $\leq 1/640$
Sick	- Dogs with no clinical signs or clinicopathologic abnormalities with positive parasitological test and negative serology
	- Dogs with consistent clinical signs and/or clinicopathologic abnormalities with positive serology $\geq 1/320$
Removed from study	- Dogs with consistent clinical signs or clinicopathologic abnormalities with positive qualitative serology or serology result of 1/160 that had their medical records reviewed by internal medicine board-certified authors and considered sick
	- Dogs not fitting in any of those previous categories

values adjusted using the False Discovery Rate (FDR) method (Benjamini and Hochberg, 1995). For the continuous variables (age, weight and duration of exposure), the F-test in one-way ANOVA was used for global comparison (Scheffe, 1959). In case significant differences were found for a given variable, the multiple comparisons test with the Bonferroni method was used for comparison of every pair of preventive measures group.

For comparison of effectiveness and occurrence of adverse events associated with the different preventive measures, chi-square test was used for global comparison. In case of significance, Fisher test was used for comparison of every pair of preventive measures group, with the p-values adjusted using the FDR method. The analysis of effectiveness was initially performed considering the three outcome groups as healthy, infected and sick. Subsequently, the analysis was repeated merging the healthy and infected groups and comparing them with the sick group. The efficacy of the preventive measures was calculated using the standard efficacy calculation:  $(\% \text{ sick controls} - \% \text{ sick with preventive measure}) / \% \text{ sick control} * 100$ . The group with no preventive measures applied was used as control.

The effectiveness of the preventive measures was also compared by binary logistic regression (Agresti, 2007; Hosmer and Lemeshow, 2000). For this analysis, the healthy and infected groups were again merged together and compared with the sick group. The independent variables included the preventive measures used and those variables found not to be homogeneously distributed across groups. Likelihood ratio test was used to test the significance of the preventive measure (Agresti, 2007).

### 3. Results

#### 3.1. Study population

A total of 1693 dogs were initially included in the study. Of these, 7 dogs were excluded because they could not be properly classified at the end of the study. Also, 39 dogs were excluded because serological tests had been performed less than six months from the initial testing. Therefore, 1647 dogs remained in the study. The study population included 838 (51.1%) males and 800 (48.9%) females, which ranged from 0.5 to 18 years (mean  $6.4 \pm 3.2$  years) of age. There were 1177 (72.7%) purebred dogs and 443 (27.3%) crossbreeds, varying in weight from 1.5 to 78 kg (mean  $20.0 \pm 13.5$  kg). One hundred and twenty three breeds were reported. Breeds with more than 50 individuals included were Yorkshire Terrier (118, 7.2%), Labrador Retriever (92, 5.6%), German Shepherd (83, 5.0%), Golden Retriever (77, 4.7%), French Bulldog (60, 3.6%), Boxer (57, 3.5%), and Bichon Maltese (57, 3.5%). Some signalment information was not reported for a few dogs (sex in 9 dogs, age in 7 dogs, breed in 27 dogs and weight in 42 dogs). Duration of exposure extended from 6 to 52 months, with a mean of  $23.1 \pm 11.6$  months. Dogs were assessed by their veterinarians as having a high risk of exposure in 70.8% of cases, and low risk in 29.2%.

#### 3.2. Test results and clinical classification

Initial serology was negative in all cases, and it was performed with a qualitative test in 885 (53.7%), enzyme-linked immunosorbent assay (ELISA) in 499 (30.3%), indirect immunofluorescence antibody test (IFAT) in 139 (8.5%), direct agglutination test (DAT) in 101 (6.1%), and other methods not specified in 23 dogs (1.4%). No dogs had clinical signs nor clinicopathologic abnormalities at this point, as required by inclusion criteria.

At the time of the second serological testing, 1477 (89.7%) dogs were categorized as healthy, 91 (5.5%) infected and 79 (4.8%) sick for leishmaniasis. In the overall population, final serological testing was performed with a qualitative test in 722 (43.9%), ELISA in 696 (42.3%), IFAT in 134 (8.1%), DAT in 76 (4.6%), and other methods not specified in 19 cases (1.1%). For the 170 dogs considered sick or infected, final

serology test was performed with a qualitative test in 5 (2.9%), ELISA in 119 (30.0%), IFAT in 43 (25.3%), DAT in 2 (1.2%), and other methods not specified in 1 case (0.6%).

Clinical signs consistent with leishmaniasis were present in 72/79 (91.1%) of sick dogs. The 7 sick dogs with no clinical signs had only bloodwork abnormalities. The most common clinical signs included were weight loss (58/79, 73%), exfoliative dermatitis (55/79, 70%), generalized lymphadenopathy (47/79, 59%), erosive-ulcerative dermatitis (29/79, 37%), lethargy (28/79, 35%), poor appetite (24/79, 30%), and pale mucous membranes (24/79, 30%). Clinicopathologic abnormalities consistent with leishmaniasis were present in 65/79 (82.3%) of sick dogs. The most common abnormalities were hyperglobulinemia (63/79, 80%), hypoalbuminemia (48/79, 60%), non-regenerative anemia (40/79, 51%), thrombocytopenia (25/79, 32%), azotemia (24/79, 30%), and proteinuria (20/79, 25%). Parasitological confirmation of infection was reported in only 20 cases, 10 cases by PCR and 10 cases by direct cytological observation of *Leishmania* amastigotes in bone marrow, lymph nodes or skin lesions preparations.

#### 3.3. Use of preventive measures

From the total population ( $n = 1647$ ), 176 dogs (10.7%) did not receive any preventive measure (NONE group), 751 dogs (45.7%) received only repellents (REP group), 69 dogs (4.2%) were solely vaccinated (VACC group), 37 dogs (2.3%) were treated with domperidone only (DOMP group) and the rest of the population received combinations of the above, including repellents + vaccination (R + V group) in 378 (23.0%), repellents + domperidone (R + D group) in 186 (11.3%), vaccination + domperidone (V + D group) in 44 (2.7%), and repellents + vaccination + domperidone (R + V + D group) in 3 (0.2%). Overall, repellents were applied in 1318 (80.2%) dogs, 494 (30.0%) dogs were vaccinated, and 270 (16.4%) dogs received domperidone. The type of repellents applied varied and included collar in 369 cases (28.0%), spot-on product in 448 cases (34.0%), and the use of collar combined with spot-on product in 501 cases (38.0%). The exact brand name of the repellents used was not specified. All dogs vaccinated used the same commercial vaccine<sup>2</sup>, as it was the only vaccine registered in Europe during the study period. The same commercially available product was used in all dogs receiving domperidone<sup>3</sup>.

The different preventive treatment groups were assessed for homogeneity of the demographic variables (age, gender, breed, weight, duration of exposure, and risk of exposure). No statistically significant differences were detected among groups regarding gender ( $p = 0.069$ ) and breed ( $p = 0.539$ ). However, significant differences were detected regarding age, weight, duration and risk of exposure ( $p < 0.001$ ) (Table 2). Since these variables could affect the risk of infection, they were included in the logistic regression analysis performed during the efficacy analysis in order to take into account their possible influence on the results (see section 3.4 below).

#### 3.4. Efficacy assessment of preventive measures

Results of the clinical classification for canine leishmaniasis (healthy, infected and sick) in the different preventive treatment groups are presented in Table 3, and the associated statistical analysis results are described in Table 4. The incidence of clinical leishmaniasis during the study period was 4.8% in the overall population, and 12.5% in the group with no preventive treatment applied (NONE group). All preventive measures studied resulted in a decreased incidence of leishmaniasis, and the distribution among categories (healthy, infected and sick) was significantly different between the NONE group and all the others. The R + V + D group was not included in the statistical analysis due to its low number of dogs. The distribution in the REP group was significantly different from all the other groups except for DOMP. The distribution in the VACC group differed significantly from the other groups, but not from V + D. The distribution in the V + D group

**Table 2**  
Homogeneity of preventive treatment groups regarding age, weight, duration and risk of exposure.

Preventive measures	Age (years)	Weight (kg)	Duration of exposure (months)	Risk of exposure (months)	
	Mean ± SD	Mean ± SD	Mean ± SD	High risk N (%)	Low risk N (%)
NONE	7.2 ± 3.5 <sup>ad</sup>	19.9 ± 12.5	21.0 ± 10.3 <sup>ad</sup>	148 (84.6%) <sup>abcd</sup>	27 (15.4%)
REP	6.6 ± 3.3 <sup>b</sup>	19.4 ± 13.3 <sup>c</sup>	22.5 ± 11.4 <sup>bc</sup>	516 (69.7%) <sup>ae</sup>	224 (30.3%)
VACC	6.1 ± 2.5	24.7 ± 14.1 <sup>a</sup>	24.6 ± 12.3	45 (66.2%) <sup>b</sup>	23 (33.8%)
DOMP	7.5 ± 3.6 <sup>c</sup>	17.3 ± 14.0	20.7 ± 8.6 <sup>f</sup>	22 (61.1%) <sup>c</sup>	14 (38.9%)
R + V	5.7 ± 3.0 <sup>abc</sup>	22.4 ± 14.7 <sup>bc</sup>	25.9 ± 11.9 <sup>abc</sup>	282 (75.4%) <sup>f</sup>	92 (24.6%)
R + D	6.1 ± 3.0 <sup>d</sup>	16.0 ± 11.2 <sup>ab</sup>	20.1 ± 11.4 <sup>cg</sup>	99 (54.7%) <sup>def</sup>	82 (45.3%)
V + D	5.9 ± 3.0	20.9 ± 13.6	28.7 ± 12.7 <sup>defg</sup>	32 (74.4%)	11 (25.6%)
R + V + D*	6.4 ± 3.2	20.0 ± 13.5	23.1 ± 11.6	3 (100%)	0 (0%)
p-value	< 0.001	< 0.001	< 0.001	< 0.001	

Within each column (age, weight, duration of exposure and risk of exposure), superscript letter indicate a pair of groups (NONE, REP, VACC, DOMP, R + V, R + D, V + D) that were statistically different. (p < 0.05).

\* The R + V + D group was not considered in the statistical analysis due to its low number of dogs (n = 3). SD, Standard deviation. NONE, group with no preventive applied; REP, repellents alone; VACC, vaccination alone; DOMP, domperidone alone; R, repellents; V, vaccination; D, domperidone.

**Table 3**  
Clinical classification for canine leishmaniasis in the different preventive treatment groups.

Preventive treatment groups	N (%)	Clinical classification of canine leishmaniasis		
		Healthy N (%)	Infected N (%)	Sick N (%)
NONE	176 (10.7%)	147 (83.5%)	7 (4.0%)	22 (12.5%)
REP	751 (45.7%)	695 (92.5%)	22 (2.9%)	34 (4.5%)
VACC	69 (4.2%)	51 (73.9%)	11 (15.9%)	7 (10.1%)
DOMP	37 (2.3%)	37 (100%)	0 (0%)	0 (0%)
R + V	378 (23.0%)	324 (85.7%)	39 (10.3%)	15 (4.0%)
R + D	186 (11.3%)	182 (97.9%)	3 (1.6%)	1 (0.5%)
V + D	44 (2.7%)	36 (81.8%)	8 (18.2%)	0 (0%)
R + V + D*	3 (0.2%)	2 (66.7%)	1 (33.3%)	0 (0%)
TOTAL	1644 (100%)	1474 (89.7%)	91 (5.5%)	79 (4.8%)

\* The R + V + D group was not considered in the statistical analysis due to its low number of dogs (n = 3). NONE, group with no preventive applied; REP, repellents alone; VACC, vaccination alone; DOMP, domperidone alone; R, repellents; V, vaccination; D, domperidone.

**Table 4**  
Pair comparisons between groups from Table 3 using the Fisher test with p-values adjusted by the FDR method. Significant values (p < 0.05) are highlighted in bold.

Groups	REP	VACC	DOMP	R + V	R + D	V + D
NONE	<b>0.0014</b>	<b>0.019</b>	<b>0.033</b>	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
REP		< <b>0.001</b>	0.479	< <b>0.001</b>	<b>0.019</b>	< <b>0.001</b>
VACC			<b>0.0032</b>	<b>0.0453</b>	< <b>0.001</b>	0.129
DOMP				0.068	1	<b>0.016</b>
R + V					< <b>0.001</b>	0.200
R + D						< <b>0.001</b>

NONE, group with no preventive applied; REP, repellents alone; VACC, vaccination alone; DOMP, domperidone alone; R, repellents; V, vaccination; D, domperidone.

differed from the groups that were not vaccinated. No differences were detected between DOMP group and REP, R + V and R + D groups.

The 751 dogs that received repellents as the only preventive treatment were further classified depending on the type of repellent used (collar, spot-on, or combination of both) (Table 5). The distribution of healthy, infected and sick categories did not differ among groups based on the type of repellent used (p = 0.64).

The analysis of efficacy was repeated comparing sick dogs with the

**Table 5**  
Clinical classification for canine leishmaniasis in dogs receiving different types of repellents.

Type of repellent used	N (%)	Clinical classification of canine leishmaniasis		
		Healthy N (%)	Infected N (%)	Sick N (%)
Collar	185 (246%)	168 (90.8%)	5 (2.7%)	12 (6.5%)
Spot-on	252 (33.6%)	234 (92.9%)	7 (2.8%)	11 (4.3%)
Collar + Spot-on	314 (41.8%)	293 (93.3%)	10 (3.2%)	11 (3.5%)
TOTAL	751 (100%)	695 (92.6%)	22 (2.9%)	34 (4.5%)

rest of the population, clustering healthy and infected dogs together in the same group. This was performed for two reasons. Firstly, all groups that included vaccination as a preventive measure had a higher incidence of infection than any other group, even higher than the group where no preventive treatment was applied (VACC 15.9%, R + V 10.3%, V + D 18.2% versus 4.0% in the NONE group). Because this could have been a consequence of misclassification of these dogs due to interference of vaccine antibodies, it was decided to repeat the analysis including these dogs in the healthy group. And secondly, comparing only two groups allowed more meaningful interpretations of the results to evaluate the efficacy of the preventive measures studied among groups. In this second analysis, significant differences among groups could directly correlate with increased or decreased numbers of sick dogs, while in the first analysis significant differences correlated with different distribution among the three categories but not necessarily a difference in the sick category. Because ultimately the final aim in efficacy is the prevention of disease development, and because vaccination and domperidone have no effect in preventing infection, the authors agreed that the results of the comparison between healthy + infected versus sick dogs would reflect more accurately the efficacy sought by preventive measures in the field population.

Results of the clinical classification for canine leishmaniasis (healthy + infected versus sick) in the different preventive treatment groups are presented in Table 6 with the associated calculated efficacies. The lowest incidence of illness was observed in the DOMP group and V + D group with no sick dogs, followed by R + D with 0.5%, R + V with 4.0%, REP with 4.5%, VACC with 10.1%, and finally dogs in the NONE group had an incidence of 12.5%. Statistical differences using the Fisher test between pair groups are represented in Table 7.

A binary logistic regression was used to take into consideration the different distribution of age, weight, duration and risk of exposure among groups, and results are depicted in Table 8. All preventive measures resulted in a significantly lower incidence of sick dogs

**Table 6**  
Clinical classification of canine leishmaniasis and preventive measures used, after merging healthy and infected dogs.

Preventive measures groups	N (%)	Clinical classification of leishmaniasis		Calculated efficacy (%)
		Healthy + Infected N (%)	Sick N (%)	
NONE	176 (10.7%)	154 (87.5%)	22 (12.5%)	
REP	751 (45.7%)	717 (95.5%)	34 (4.5%)	64.0%
VACC	69 (4.2%)	62 (89.9%)	7 (10.1%)	19.2%
DOMP	37 (2.3%)	37 (100%)	0 (0%)	100%
R + V	378 (23.0%)	363 (96.0%)	15 (4.0%)	68.0%
R + D	186 (11.3%)	185 (99.5%)	1 (0.5%)	96.0%
V + D	44 (2.7%)	44 (100%)	0 (0%)	100%
R + V + D*	3 (0.2%)	3 (100%)	0 (0%)	100%
TOTAL	1644 (100%)	1565 (95.2%)	79 (4.8%)	

\* The R + V + D group was not considered in the statistical analysis due to its low number of dogs (n = 3). NONE, group with no preventive applied; REP, repellents alone; VACC, vaccination alone; DOMP, domperidone alone; R, repellents; V, vaccination; D, domperidone.

**Table 7**  
Pair comparisons between groups from Table 6 using the Fisher test with p-values adjusted by the FDR method. Significant values (p < 0.05) are highlighted in bold.

Groups	REP	VACC	DOMP	R + V	R + D	V + D
NONE	<b>0.003</b>	1	0.068	<b>0.0035</b>	< <b>0.001</b>	<b>0.044</b>
REP		0.187	0.696	1	<b>0.044</b>	0.541
VACC			0.217	0.171	<b>0.0037</b>	0.129
DOMP				0.696	1	1
R + V					0.095	0.696
R + D						1

NONE, group with no preventive applied; REP, repellents alone; VACC, vaccination alone; DOMP, domperidone alone; R, repellents; V, vaccination; D, domperidone.

**Table 8**  
Pair comparisons between groups from Table 6 using binary logistic regression, taking into account the variables age, weight, duration of exposure, and risk of exposure. Significant values (p < 0.05) are highlighted in bold.

Groups	REP	VACC	DOMP	R + V	R + D	V + D
NONE	<b>0.001</b>	0.331	<b>0.003</b>	< <b>0.001</b>	< <b>0.001</b>	< <b>0.001</b>
REP		<b>0.042</b>	0.085	0.772	< <b>0.001</b>	0.081
VACC			<b>0.008</b>	<b>0.024</b>	< <b>0.001</b>	<b>0.006</b>
DOMP				0.078	1	1
R + V					<b>0.003</b>	0.066
R + D						1

NONE, group with no preventive applied; REP, repellents alone; VACC, vaccination alone; DOMP, domperidone alone; R, repellents; V, vaccination; D, domperidone.

compared to not giving any measure, except for the administration of vaccination alone. Use of repellents alone was significantly better than vaccination, but was significantly less effective than the combination of repellents and domperidone. Vaccination alone resulted in a higher incidence of sick dogs than any other preventive measure or combination. Overall, the group that performed statistically better was the combination of repellents and domperidone.

### 3.5. Adverse events

Adverse events were reported in 72 (72/1394, 5.2%) dogs receiving

**Table 9**  
Occurrence of adverse events across groups based on the preventive measure used.

Groups	N	Adverse events	
		Yes	No
NONE	161	0 (0%)	161 (100%)
REP	694	2 (0.3%)	692 (99.7%)
VACC	67	6 (9.0%)	61 (91.0%)
DOMP	37	1 (2.7%)	36 (97.3%)
R + V	369	47 (12.7%)	322 (87.3%)
R + D	180	2 (1.1%)	178 (98.9%)
V + D	44	11 (25.0%)	33 (75.0%)
R + V + D*	3	3 (100%)	0 (0%)
TOTAL	1555	72 (4.6%)	1483 (95.4%)

\* The R + V + D group was not considered in the statistical analysis due to its low number of dogs (n = 3). NONE, group with no preventive applied; REP, repellents alone; VACC, vaccination alone; DOMP, domperidone alone; R, repellents; V, vaccination; D, domperidone.

some preventive measure. The occurrence of adverse events across the different groups is detailed in Table 9, and the associated statistical results are described in Table 10. The V + D group had the highest rate of adverse events with 25% followed by R + V with 12.7%, and they were significantly different from the other groups. The VACC group had 9% occurrence of adverse events, and it was significantly higher than NONE (0%), REP (0.3%) and R + D (1.1%). Finally, DOMP with 2.7% was not significantly different from NONE, REP and R + D groups.

Adverse events were associated with vaccination in 63 cases, repellents in 5 cases, and domperidone in 2 cases. In 2 cases, it was not possible to attribute the adverse events to a specific measure. The most common adverse events reported were lethargy (39 cases, 2.8%), pain on palpation at the inoculation site (27, 1.9%), cutaneous inflammation at the inoculation site (24, 1.7%), mild anaphylactic reaction (19, 1.4%), hyperthermia (15, 1.1%), erythema (7, 0.5%), severe anaphylactic reaction (5, 0.4%) and diarrhea (5, 0.4%).

## 4. Discussion

In this large-scale study, all preventive measures instituted, except for vaccination alone, were effective in decreasing the incidence of developing clinical leishmaniasis. The use of repellents alone and the combination of repellents and domperidone were the most effective preventive treatments. Overall, there was a low incidence of adverse events (4.6%), and these were most often associated with vaccination.

The incidence of disease in unprotected dogs in our study was 12.5% during a mean follow-up period of almost two years. Although not directly comparable, this incidence is similar to previously reported *Leishmania* seroprevalences in the Mediterranean area (Couto et al., 2010; Franco et al., 2011; Maia et al., 2015; Miró et al., 2013; Solano-Gallego et al., 2009).

**Table 10**  
Pair comparisons between groups from Table 9 using the Fisher test with p-values adjusted by the FDR method. Significant values (p < 0.05) are highlighted in bold.

Groups	REP	VACC	DOMP	R + V	R + D	V + D
NONE	1	<b>0.0013</b>	0.213	< <b>0.001</b>	0.538	< <b>0.001</b>
REP		< <b>0.001</b>	0.192	< <b>0.001</b>	0.231	< <b>0.001</b>
VACC			0.483	0.561	<b>0.01</b>	<b>0.0476</b>
DOMP				0.146	0.483	<b>0.0091</b>
R + V					< <b>0.001</b>	0.0548
R + D						< <b>0.001</b>

NONE, group with no preventive applied; REP, repellents alone; VACC, vaccination alone; DOMP, domperidone alone; R, repellents; V, vaccination; D, domperidone.

In the present study, 89.1% of dogs were receiving one or more preventive treatments against leishmaniasis. Similar results have been published in studies performed in south European countries (Bourdeau et al., 2014; Goyena et al., 2016; Lladró et al., 2017; Matos et al., 2015; Pereira et al., 2016), where 92–98% of dogs were receiving some ectoparasite control, although in those studies not all dogs were receiving all year round protection. There is probably a selection bias of dogs regularly attending the veterinarian that might be more prone to have preventive treatments applied (Goyena et al., 2016). In our study, 10.9% of dogs were not under any prophylactic treatment, despite being regularly tested for leishmaniasis at the veterinarian. This may be due to poor owner compliance, which remains an ongoing challenge for veterinarians. Nevertheless, this emphasizes the need for the veterinary profession to be more pro-active in communicating the benefits and importance of preventive measures for canine leishmaniasis to clients.

The incidence of clinical leishmaniasis in dogs in which only repellents were applied was 4.5%, with a calculated repellent efficacy of 64.0%. This efficacy is generally lower than what has been previously reported in the literature, and it is more remarkable considering that 41.8% of dogs were receiving the combination of a collar and a spot-on product. Efficacy using a deltamethrin collar varies from 61.8 to 84% (Brianti et al., 2016; Ferroglio et al., 2008; Foglia Manzillo et al., 2006) while more recently, the efficacies reported for a 10% imidacloprid + 4.5% flumethrin collar were 88.3–100% (Brianti et al., 2016, 2014; Otranto et al., 2013). A combination of 10% imidacloprid + 50% permethrin as a spot-on formulation is highly effective, with 89–100% preventive effect (Otranto et al., 2010, 2007). Because the name of the exact repellent formulation administered was not specified in our study, it is not possible to analyze the different efficacies for each particular product or to discern if low-end products were used. Another possible explanation for the perceived decreased efficacy is poor owner adherence to manufacturer recommendations (Coles and Dryden, 2014). Although this was specifically an inclusion criterion in our study, it is possible that owners were not 100% compliant. A recent study in the United States (Lavan et al., 2017) showed that although all veterinarians were actively recommending 12 months of ectoparasite prevention, only 62% of owners followed this recommendation, and it was estimated that the duration of the actual ectoparasite control was only 6.1 months. Further analyses of efficacy were performed for the different types of repellents used (collar, spot-on, or both). Although the combination of collar and spot-on resulted in a lower percentage of sick dogs compared to the use of either products alone, the percentage of infected dogs was similar among groups, with no statistical differences among the three groups. These results were unexpected, as the combination of products should provide a synergistic effect, as suggested in a previous study (Goyena et al., 2016). These results highlight the importance of field studies when assessing efficacy treatments since external factors other than preventive treatment efficacy might affect the performance of these products.

No dogs in which domperidone was administered as the sole preventive treatment got sick, deriving in a calculated efficacy of 100%. In addition, the incidence of overt disease in the domperidone group was significantly lower than in groups where vaccination or no treatment was applied, but was not significantly different from the other groups where repellents were used. This lack of significance is probably due to a type II statistical error, since only 37 dogs used solely domperidone. However, although the number of dogs on domperidone was low, an additive positive effect of this drug on preventing patent clinical leishmaniasis is likely, given the significantly lower incidence of disease observed in dogs receiving repellents as well as domperidone, compared to those only on repellents. These results are similar to those reported in the literature. The preventive effect of domperidone published in a previous study was 80% during the first 12 months, and 77% throughout the 21-month duration of the study (Sabaté et al., 2014). Moreover, domperidone has proved effective in treating dogs with mild forms of the disease, therefore possibly providing additional benefits to

animals who become infected (Gómez-Ochoa et al., 2009; Passos et al., 2014).

Vaccination was commonly used in our study but failed to show a significant effect in preventing development of disease compared to unprotected dogs. Unfortunately, vaccine antibodies interference may have resulted in clinical misclassification of vaccinated dogs, especially in the “infected” category. Vaccination with CaniLeish® interferes with antibodies measured by serological assays for *Leishmania* (Solano-Gallego et al., 2017). Although these antibodies can persist for 4–12 months after the primary course of vaccination, they tend to be low (Starita et al., 2016). In a study of blood donor dogs followed over 2 years after vaccination with CaniLeish®, most dogs developed low antibody titers and only 2/27 dogs developed an IFAT titer of 1/320 after 42 days of initial vaccination (Starita et al., 2016). Therefore, although probably many vaccinated dogs considered in the “infected” category were wrongly classified, it is less likely that vaccinated dogs in the “sick” category were misclassified. Ideally, the classification of dogs should take into account not only antibody titers but also parasitological confirmation (Solano-Gallego et al., 2017; Starita et al., 2016). Unfortunately, given the retrospective nature of the study, this information was not available in most cases. Another limitation in determining vaccine efficacy was the small number of dogs receiving vaccination compared with those receiving repellents that might have limited the power to detect differences.

Consequently, although the results on efficacy of vaccination in this study are discouraging, these results should be interpreted cautiously and need to be confirmed with additional studies. Literature regarding efficacy of vaccination in Europe is scarce. Oliva et al reported in 2014 an efficacy of 68.4% in the prevention of development of clinical leishmaniasis using CaniLeish® in Beagle dogs placed in two endemic areas. A recent study on the recombinant protein Q based vaccine (Letifend®, Leti) has shown an efficacy of 72% (Fernández Cotrina et al., 2018), but our study was performed before its release and therefore, none of the dogs in our vaccination groups received this newer vaccine. In the other hand, another study (Brianti et al., 2016) failed to detect a difference in incidence of active infection between vaccinated and non-vaccinated dogs, although problems with sample size and methodology might have interfered with the results, especially considering that immunoprophylaxis methods of prevention have no effect on the incidence of parasite infection (Maroli et al., 2010; Miró et al., 2017).

Altogether, the combination of repellents and domperidone outperformed the other preventive measures, with a calculated efficacy of 96%. In our study, efficacy was assessed by looking at the incidence of sick dogs for all preventive measures. However, consideration must be taken to the fact that preventive strategies act at different levels. For instance, repellents avoid the bite of sand flies and therefore, prevent infection with *Leishmania*. On the other hand, while the use of immunoprophylactic methods such as vaccination or domperidone will not prevent infection, they should be effective at decreasing the number of dogs developing the disease (Maroli et al., 2010; Miró et al., 2017). In the literature, most studies evaluate the efficacy of one particular product and reported efficacies are based on prevention of infection or disease depending on the measure evaluated (Brianti et al., 2014; Grimaldi et al., 2017; Oliva et al., 2014; Otranto et al., 2013, 2010; Regina-Silva et al., 2016; Sabaté et al., 2014). There is only one report in which different preventive strategies (2 collars and 1 vaccine) were evaluated in the same study and efficacies were also compared directly (Brianti et al., 2016). Our study is the first to compare different strategies and the combined use of these strategies on dogs on the field. Although expert recommendations include the combination of different preventive strategies (Solano-Gallego et al., 2011), no published literature has assessed these combinations so far (Otranto and Dantas-Torres, 2013). According to our results, the combination of repellents and domperidone could have an additional benefit over the sole use of repellents and therefore, it could be an interesting recommendation for

global strategy for the prevention of leishmaniasis in dogs.

Adverse events were reported in 5.2% of all cases and were significantly more common in dogs being vaccinated (9–25%). Most adverse events secondary to vaccination were mild and self-limiting, and included lethargy or signs related to the inoculation site, with only 5 cases of severe anaphylactic reaction. These results are consistent with published information on CaniLeish® safety (European Medicines Agency, 2011; Martin et al., 2014; Moreno et al., 2014; Oliva et al., 2014), where transient signs at the point of inoculation are the most commonly reported side effects. In our study, only five adverse events were attributed to the use of repellents and consisted of erythema in 3, nervousness in 1 and an anaphylactic reaction in 1. This low incidence rates supports the current literature (Lladró et al., 2017; Wylie et al., 2014). Finally, only two dogs receiving domperidone were reported to have an adverse event, which included lethargy (1 dog) and galactorrhea and pseudopregnancy (1 dog). Previous studies on the use of domperidone reported similar side effects with an incidence of 0–9% (Gómez-Ochoa et al., 2012; Sabaté et al., 2014). Due to the retrospective nature of the study, it was possible that some adverse events had been under-reported or missed, especially with regard to preventive treatments administered at home. Nevertheless, this study confirms the general safety of the available preventive measures used against canine leishmaniasis.

The present study has several limitations. Due to the retrospective nature of the study, the groups were not homogeneous regarding the number of dogs included or their demographics. Specific statistics were used to account for the different demographics in the groups, but the low sample size in some groups (especially domperidone) might have precluded finding some statistical differences. Another limitation was the possible interference of vaccine antibodies with the clinical classification of canine leishmaniasis as discussed earlier. Unfortunately, cytology or PCR of bone marrow or lymph node are not routinely performed by veterinarians in private practice, which would have been necessary to determine the infectious status in vaccinated dogs. This limitation has also been observed in questionnaire-based studies in different endemic areas of southern Europe (Ballart et al., 2013; Bourdeau et al., 2014; Lladró et al., 2017). However, for the reasons mentioned previously and the classification criteria used, it is unlikely that sick dogs were incorrectly classified. Another limitation of our study was the widespread use of qualitative serological tests as a screening tool for the diagnosis of canine leishmaniasis. Suboptimal sensitivity has been reported for a number of these commercially available tests (Athanasίου et al., 2014; Solano-Gallego et al., 2017, 2014), and might have resulted in seropositive dogs being included initially in the study or infected dogs not being detected at the end of the study. Conversely, specificity of these tests is reported to be high and also most veterinarians did confirm a positive qualitative result with a quantitative serological method, resulting in robust results in the group of sick dogs.

## 5. Conclusion

In conclusion, this is the first field large-scale study investigating the efficacy and safety of the preventive measures used routinely against leishmaniasis in client-owned dogs in Europe. Most preventive strategies used, with exception of vaccination alone, had benefit in preventing the development of clinical leishmaniasis over not applying any preventive. According to our results, the use of repellents is effective and the use of domperidone seems to provide a synergistic effect in preventing clinical leishmaniasis. The role of vaccination and its combination with other preventive strategies needs further study. The fact that a high percentage of dogs had no preventive measure applied despite living in endemic regions highlights the importance of client education in prevention of canine leishmaniasis.

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## Conflict of interest

Two of the authors (JH and CM) are currently employed at Ecuphar veterinaria SLU, and assisted with study design and review of the manuscript.

## Notes

<sup>1</sup>Statgraphics Centurion XVII.

<sup>2</sup>CaniLeish, Virbac.

<sup>3</sup>Leisguard 5 mg/ml Oral Suspension for dogs, Ecuphar veterinaria SLU.

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