Revista da Sociedade Brasileira de Medicina Tropical 49(1):107-111, Jan-Feb, 2016
http://dx.doi.org/10.1590/0037-8682-0187-2015

Short Communication

Predictive factors for *Leishmania infantum* infection in dogs examined at a veterinary teaching hospital in Teresina, State of Piauí, Brazil

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ABSTRACT

Introduction: In Brazil, culling of seropositive dogs is one of the recommended strategies to control visceral leishmaniasis. Since infectiousness is correlated with clinical signs, control measures targeting symptomatic dogs could be more effective. **Methods:** A cross-sectional study was carried out among 1,410 dogs, predictive models were developed based on clinical signs and an indirect immunofluorescence antibody test. **Results:** The validated predictive model showed sensitivity and specificity of 86.5% and 70.0%, respectively. **Conclusions:** Predictive models could be used as tools to aid control programs in focusing on a smaller fraction of dogs contributing more to infection dissemination.

Keywords: Canine. Control measures. Visceral leishmaniasis.

Visceral leishmaniasis (VL) is a severe disease affecting thousands of people worldwide, mainly those living in poverty in developing countries[1]. In Brazil, the disease is caused by the protozoan parasite *Leishmania infantum* (syn = *Leishmania chagasi*), transmitted by the bites of female sandflies from the genus *Lutzomyia*, and dogs are considered the main source of infection in urban areas.

Infected dogs may not exhibit clinical signs, but many will develop signs of the disease during the course of infection[2]. Both clinically and subclinically affected dogs may transmit *Leishmania* to sandflies, but clinically affected dogs transmit the disease much more efficiently[2] [3].

As dog infectiousness is correlated with clinical signs[3], the development of a predictive system for canine visceral leishmaniasis (CVL) combining serological results with clinical information might be an alternative for improving the effectiveness of VL control strategies.

We report a cross-sectional study carried out among 1,410 dogs (from 1 month to 13 years of age) from January 2003 to December 2004. These animals, brought by their owners, were examined by veterinarians at the university veterinary hospital of the Federal University of Piauí (FUFPI) and sent to the Animal Health Laboratory (FUFPI) for serological and parasitological testing.

A diagnosis of CVL (the primary outcome measure) was confirmed when *Leishmania* spp. amastigotes could be detected in bone marrow aspirates, lymph node aspirates, or skin samples. Bone marrow biopsy was performed on all dogs ≥ 3 months of age, while lymph node smears were obtained only from those with lymphadenopathy, and skin samples were obtained from those showing skin lesions.

Serological testing was performed by means of an indirect immunofluorescence antibody test (IFAT) using a canine leishmaniasis kit supplied by Bio-Manguinhos, FIOCRUZ, Rio de Janeiro, Brazil. Five veterinarians were responsible for clinical evaluations of the dogs upon arrival at the hospital, and the findings were recorded on a standardized clinical evaluation form.

The predictive variables considered were based on clinical signs of the disease (presence or absence), sex (male/female), age (< 1, 1, 2, 3, 4-5, and > 5 years; used as an ordinal variable in the analyses) and IFAT results. We used a split-sample validation approach in which the original dataset was divided into two groups: the *test sample* (dogs evaluated in 2003, n = 713) to develop the models, and the *validation sample* (dogs evaluated in 2004, n = 697) for model validation[4].

To develop predictive models, we initially employed simple logistic regression models to estimate odds ratios (ORs) and respective 95% confidence intervals. Variables showing a

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Received 15 June 2015
Accepted 16 September 2015

univariate association with CVL at a p-value ≤ 0.20 were selected for the multivariate analyses. Only variables with an association with a p-value ≤ 0.05 remained in the final model. Three predictive models were developed: one with only demographic and clinical variables (the *clinical model*), one based only on IFAT results (the *IFAT model*), and one with both IFAT results and demographic and clinical variables (the *clinical + IFAT model*).

A predictive model based on a scoring system, with points allocated to each predictive variable, was created from each of the three final regression models run on the test sample[5]. A final score for each model was obtained through the sum of the points attributed to the presence of each predictive variable that remained in the final model and from the results of the IFAT. These models were then validated using the validation sample.

The area under the receiver operating characteristic curve (auROC), sensitivity, and specificity were used to evaluate the performances of the models. Sensitivity and specificity were obtained considering a predicted probability of CVL of 40% (≥ 40% indicating the presence of CVL), a value close to the prevalence of CVL in both the test and validation samples (38.2% in 2003 and 43.6% in 2004). The calibration of the model with the higher discriminatory ability was evaluated using the Hosmer-Lemeshow statistic[6]. Statistical analyses were performed using Stata 9.2 (STATA Corp., College Station, TX, USA).

From all predictive demographic and clinical variables selected for multivariate analyses, only variables listed in **Table 1** and **Table 2** remained significantly associated with CVL in the final clinical model.

Table 3 shows the scores for each clinical variable and IFAT result obtained from models developed from the test sample. Considering a predicted probability of CVL of 40%, the cutoff for the scoring system indicating CVL was ≥ 2 for the clinical model, and ≥ 9 for the clinical + IFAT model. For the IFAT model, the cutoff was ≥ 2, corresponding to an IFAT reaction ≥ 1:80.

The scoring system was then applied to the validation sample to evaluate its performance, and the following results were obtained for the clinical model, IFAT model, and IFAT + clinical model: 1) sensitivity: 75.3%, 87.8%, and 86.5%; and 2) specificity: 65.9%, 63.6%, and 70%, respectively. The auROC curve of the clinical + IFAT model (87.9%) was significantly higher than that of the IFAT model (84.2%) (p = 0.001). Model calibration indicated no statistically significant difference between the predicted and observed outcomes for the clinical + IFAT model in the validation sample (p = 0.313).

Since both clinical status and levels of antibodies are associated with infectiousness[2] [3], the use of such a model would be in line with the idea that focused interventions oriented towards dogs that contribute more to transmission tend to be more efficient[7] [8]. Although removing only highly infectious dogs might not be sufficient to interrupt transmission, since asymptomatic dogs can also transmit *Leishmania*, it has been suggested that interventions targeting subgroups that contribute most to transmission will have a higher impact than non-targeted interventions[7].

A critical point to be highlighted is that a predictive model using IFAT might not be useful in practice, since the Brazilian Ministry of Health changed the serological test used for detecting seropositive dogs in the field. However, one may consider this an initial effort that demonstrates the potential of a control strategy focusing on more highly infectious dogs. As a matter of fact, the use of IFAT as a serological test does not invalidate

TABLE 1 - Odds ratios and 95% confidence intervals for CVL for variables retained in the final models generated from the test sample.

		Clinical model			IFAT model			Clinical + IFAT model		
Variable	**N**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
IFAT (ordinal)	713	--	--	--	2.44	2.13–2.78	< 0.001#	2.62	2.23-3.07	< 0.001#
Age (ordinal)	713	0.74	0.67-0.82	< 0.001#	--	--	--	0.76	0.67-0.86	< 0.001#
Periorbital alopecia										
no	685	1.00						1.00		
yes	28	3.94	1.48-10.44	0.006	--	--	--	3.69	1.11-12.31	0.034
Weight loss										
no	660	1.00						1.00		
yes	53	2.38	1.26-4.50	0.007	--	--	--	2.29	1.07-4.89	0.033
Skin lesions										
no	658	1.00						1.00		
yes	55	2.65	1.39-5.05	0.003	--	--	--	2.84	1.28-6.30	0.010

CVL: canine visceral leishmaniasis; **IFAT:** immunofluorescence antibody test; **OR:** odds ratios adjusted for other variables in the table; **CI:** confidence interval; #p-value for the trend test.

TABLE 2 - Odds ratios and 95% confidence intervals for CVL for variables retained in the final models generated from the test sample.

		Clinical model			IFAT model			Clinical + IFAT model		
Variable	**N**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Lesions on lips										
no	681	1.00						1.00		
yes	32	2.70	1.15–6.34	0.023	--	--	--	3.92	1.44–10.67	0.008
Lesions on the nose										
no	508	1.00						1.00		
yes	205	4.87	3.29–7.18	< 0.001	--	--	--	6.19	3.83–9.99	< 0.001
Ear lesions										
no	670	1.00						1.00		
yes	43	6.49	2.87–14.70	< 0.001	--	--	--	5.65	2.03–15.73	0.001
Lymphadenopathy										
no	532	1.00						1.00		
yes	181	1.82	1.22–2.70	0.003	--	--	--	2.32	1.42–3.78	0.001

CVL: canine visceral leishmaniasis; **IFAT:** immunofluorescence antibody test; **OR:** odds ratios adjusted for other variables in the table; **CI:** confidence interval.

TABLE 3 - Scores attributed to categories of predictive variables associated with CVL obtained from the test sample.

	Category	**Clinical model**	**IFAT model**	**Clinical + IFAT model**
Age (years)	1	-1	--	-1
	2	-2	--	-2
	3	-3	--	-3
	4–5	-4	--	-4
	> 5	-5	--	-5
Periorbital alopecia	Yes	5	--	5
Weight loss	Yes	3	--	3
Skin lesions	Yes	3	--	4
Lesions on the lips	Yes	3	--	5
Lesions on the nose	Yes	5	--	7
Ear lesions	Yes	6	--	6
Lymphadenopathy	Yes	2	--	3
IFAT	1:40	--	1	3
	1:80	--	2	6
	1:160	--	3	9
	1:320	--	4	12
	≥ 1:640	--	5	15

CVL: canine visceral leishmaniasis; **IFAT:** immunofluorescence antibody test.

the study, and actually indicates that the model may have even better performance if used with other more accurate serological tests. Therefore, the present study, being the first to use this approach for visceral leishmaniasis, contributes new ideas and opportunities for research focusing on the development of a more rational control strategy for VL in Brazil.

Other points regarding the importance of an approach focusing on clinical signs combined with serological tests should be emphasized. First, if this model was applied in practice, a dog would only be culled if it scored ≥ 9 points, which is a score difficult to obtain only on the basis of an IFAT result, unless there is IFAT positivity at a 1:160 dilution, which is much more specific than the cutoff commonly used in Brazil (1:40). This indicates the importance of clinical signs in association with serological tests to optimize disease control. Second, selecting only a fraction of seropositive dogs to cull from the environment would help increase the ability of health services to sustain control actions. For instance, using the common IFAT cutoff of 1:40, 499 dogs from the validation sample would be culled. However, in the clinical + IFAT model, only 380 would be culled, resulting in a relative decrease of 23.8% in the total population of potentially culled dogs. Finally, applying such a model in canine surveys could be useful, even if dog culling is abandoned as a public health intervention. Detecting the most infectious dogs could be used as a marker of a higher risk of transmission to humans, and could contribute to identifying areas of priority to which other types of interventions should be allocated. All of these considerations would apply broadly to any predictive model based on a combination of clinical signs and any available serological test.

A particular limitation of the study is the distinctive feature of the sample (dogs examined at a veterinary hospital), which presented a higher prevalence of canine infection than most settings in which VL transmission occur. Therefore, one would expect lower positive predictive values if any of the models was applied in field conditions.

The use of parasitological tests as a reference standard for CVL diagnosis is another potential limitation of this study. The well-known imperfect sensitivity of parasitological tests, and in particular, for asymptomatic dogs, leads to many truly infected dogs being classified as not infected (false negatives)[9] [10]. This might have contributed to underestimations of their specificities. Not considering the severity of clinical signs, but only their presence or absence, is an additional limitation, since the disease severity is directly correlated with infectiousness[11].

Chronic infections, such as those caused by *Leishmania* spp., *Trypanosoma cruzi*, *Ehrlichia canis*, and *Babesia canis*, may coexist in the same dog, and cross-reactions cannot be excluded as a source of error in this study[12]. Although serological cross-reactivity with *Ehrlichia canis* and *Babesia canis* might not be common[13] [14], the burden of ehrlichiosis and babesiosis in this population should be investigated, since the clinical presentations of both diseases might be similar to that of CVL, which would affect the specificity of the model using clinical signs in particular. The use of serological testing based on recombinant antigens could be a solution to reduce the threat of serological cross-reactions[15] [12].

Considering all the limitations described, one may view the predictive models developed and validated in our study more as examples of how to approach the problem by focusing on clinically affected animals to help improve the efficiency of VL control programs rather than to properly define a method that could be used promptly in different settings. Clinical signs together with the results of a serological test could be used to select a smaller fraction of the dog population to be culled from the environment, or alternatively, to be used as markers of high transmission areas when prioritizing interventions.

Ethical considerations

This study is part of a research proposal approved by the Ethics Committee for Research with Animals / Federal University of Piauí (026/2008).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

Partially funded by CNPq (#306267/2010-1; #483378/2010-0; #311507/2014-0).

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