Veterinary Parasitology 172 (2010) 204–213

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Canine leishmaniasis in Algeria: True prevalence and diagnostic test characteristics in groups of dogs of different functional type

Amel Adel[a,b,c], Claude Saegerman[b], Niko Speybroeck[c], Nicolas Praet[c], Bjorn Victor[c], Redgi De Deken[c], Abdelkrim Soukehal[d], Dirk Berkvens[c,*]

[a] *BP 179 Cité Malki Ben Aknoun, Alger, Algeria*
[b] *University of Liege, Faculty of Veterinary Medicine, Department of Infectious and Parasitic Diseases, Epidemiology and Risk Analysis Applied to Veterinary Sciences, Boulevard de Colonster, 20, B43b, B-4000 Liege, Belgium*
[c] *Institute of Tropical Medicine, Department of Animal Health, Nationalestraat 155, B-2000 Antwerp, Belgium*
[d] *Service Épidémiologie et Médecine Préventive au Centre Hospitalo-Universitaire de Beni-Messous, Alger, Algeria*

ARTICLE INFO

Article history:
Received 4 November 2009
Received in revised form 28 April 2010
Accepted 10 May 2010

Keywords:
Canine leishmaniasis
Leishmania infantum
Algeria
Bayesian analysis
Test characteristics
Prevalence

ABSTRACT

A Bayesian approach was used to assess the prevalence of *Canine leishmaniasis* and evaluate three serological diagnostic tests: indirect fluorescent antibody test (IFAT), direct agglutination test, and particle gel immuno-assay (PaGIA) for *Canine leishmaniasis* (CL) in Algiers. Four hundred and sixty-two dogs were involved in this study and divided in four groups according to their functional type: stray dogs, farm dogs, national guard dogs and pet dogs. The stray dog group showed the highest prevalence of leishmaniasis (11.7%), followed by the national guard dogs (9.7%) and the farm dogs (5.9%). IFAT was shown to be the most sensitive test in all groups. However, IFAT specificity was considerably lowered in the farm dog group: 65.2% versus 94.5% for the stray dogs. A considerable drop in PaGIA specificity was noted in the stray dogs group. The results of the current study demonstrate the variability of test characteristics in different situations and underline the danger of using standard values, without verifying their appropriateness for the specific purposes.

1. Introduction

Leishmaniases are visceral and cutaneous parasitic diseases, found worldwide (Dereure, 1993). Visceral human leishmaniasis (VHL) – also known as kala azar – is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and anemia. If left untreated, the fatality rate in developing countries can be as high as 100% within 2 years (WHO, 2009). *Leishmania infantum*, an intracellular protozoan, is the causal agent of the human and animal visceral leishmaniasis in the Mediterranean basin (Sideris et al., 1999). It is transmitted by the bites of female sandflies of the genus *Phlebotomus* (Phlebotominae, Diptera) (Killick-Kendrick, 1990), such as *Phlebotomus perniciosus* in Algeria (Izri et al., 1990).

Leishmaniasis is a zoonotic disease for which dogs are considered to be the chief reservoir of the parasite (Solano-Gallego et al., 2001) and are considered responsible for the persistence of VHL (Abranches et al., 1991). Currently, the annual worldwide incidence of VHL is estimated at around 500,000 cases (Desjeux, 2004). However, the World Health Organisation estimates that only a third of the new cases is officially declared.

In Algeria, VHL has been known since long to occur in the humid and sub-humid areas (Dereure, 1993). The annual number of human cases is estimated at about 400 (Benikhlef et al., 2001), with an incidence rate of 0.41 per 100,000 inhabitants. By Wilaya (Province), this incidence rate varies between 0.11 and 2.85 (Benhabyles and Boughoufalah, 2004). Harrat et al. (1995) reported an

* Corresponding author. Tel.: +32 3 2476393; fax: +32 3 2476268.
E-mail address: dberkvens@itg.be (D. Berkvens).

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doi:10.1016/j.vetpar.2010.05.009

unmistakable spread of the disease during the preceding period to areas which were until then uninfected, giving rise to cases of VHL in children who had never left the capital. These particular cases were explained by a recrudescence of *Canine leishmaniasis* (CL) in the Wilaya of Algiers. At the Institut Pasteur d'Alger, 37% of 1800 dogs presenting a strong suspicion or a risk of infection tested positive in an immunofluorescence antibody test (IFAT) (Harrat and Belkaïd, 2003).

CL is a severe, often fatal systemic disease (Ferrer et al., 1995). The clinical signs are variable and can mimic other infections (Office, 2005). More than half of the infected animals remain asymptomatic during a variable period of incubation that can range from three months to several years (Cardoso et al., 2004; Ferrer et al., 1995). They can, however, be as infective to the vector as symptomatic dogs (Molina et al., 1994). For these reasons, it is important to diagnose CL as early as possible to prevent further transmission (Schallig et al., 2002).

The direct parasitological test suffers from low sensitivity (El Harith et al., 1989) and the detection of specific anti-*Leishmania* antibodies in canine sera remains an important diagnostic tool (Schallig et al., 2002; Mettler et al., 2005). However, no gold standard test exists for either VHL or for CL (Boelaert et al., 1999a,b). Simultaneous estimation of prevalence of infection and diagnostic test characteristics has been carried out successfully when applying several diagnostic tests to every individual, using a Bayesian approach combining test results and external information, such as experimental evidence and expert opinion (Adel, 2002; Berkvens et al., 2006; Geurden et al., 2006; Lesaffre et al., 2007). This Bayesian modelling allows resolving issues related to the lack of a gold standard for *L. infantum* infection in the interpretation of serological survey data: prevalence and test characteristics were previously often incorrectly determined against parasitology, known to be poorly sensitive, as a reference test (Boelaert et al., 1999a; Dye and Vidor, 1993).

A survey, involving 462 dogs, was conducted between 2004 and 2005 in the area of Algiers. The purpose of the study was to compare three serological diagnostic tests and to assess the prevalence of the CL in this area using a multi-testing Bayesian model. This is the first Bayesian model to allow a comprehensive understanding of major differences in prevalence and test characteristics in function of the functional use of dogs for CL in Algeria.

2. Materials and methods

2.1. Animals

A random cross-sectional survey was carried out in the canine population of the Algiers area. No stratification was made according to breed, sex or age of the animal. Thus, 462 sera were randomly collected between late October 2004 and the end of June 2005. The samples were accompanied by an epidemiological questionnaire detailing the origin of the animal, its age, sex and breed, the environment in which it was kept and the eventual presence of clinical symptoms.

Four groups were defined in function of the functional use of the animal: stray dogs ($n = 218$), farm dogs ($n = 87$), national guard (Gendarmerie) dogs ($n = 92$) and pet dogs ($n = 65$).

2.2. Serology

Blood was collected from the radial vein in dry tubes. Three diagnostic tests were used: indirect fluorescent antibody test (IFAT), direct agglutination test (DAT) and a particle gel immuno-assay test (Vet-Pagiatest, PaGIA).

IFAT was performed as described by Vercammen and De Deken (1996) and Mancianti and Meciani (1988), using promastigotes of *L. infantum* as the antigen. Anti-*Leishmania* antibodies were detected using rabbit anti-dog Immunoglobulin G (IgG) secondary antibodies (whole molecule) conjugated to fluorescein isothiacyanate (FITC) (Sigma–Aldrich, St Louis, MO, USA). A cut-off dilution of 1:128 was used (Abranches et al., 1983, 1991). It should be noted that this cut-off value is different from the one traditionally applied in Algeria, which is 1:80.

DAT was performed with the commercial kit supplied by the Institute of Tropical Medicine (Antwerp), using a cut-off value of 1:320 (El Harith et al., 1989; Boelaert et al., 1999a). The DAT/CL antigen is a freeze-dried suspension of trypsin-treated, fixed and stained culture from promastigotes of *L. infantum*. In the presence of antibodies to *Leishmania* (IgG), the DAT/CL antigen is agglutinated.

PaGIA is a commercial gel-based rapid antibody test (DiaMed AG, Cressier sur Morat, Switzerland) that was carried out according to the instructions of the supplier (Mettler et al., 2005).

The test is based on the rK39, a recombinant protein with a repetitive epitope closely conserved among members of the *Leishmania donovani* complex (Reed, 1995).

2.3. Statistical analysis

The interpretation of results of diagnostic tests require the inclusion of the so-called external information: the data at hand (test results) do not contain any information about test sensitivity and test specificity. Inclusion of this information is essentially a subjective process. For example, the person interpreting the test results may decide to use the values of test sensitivity and test specificity as supplied by the producer of the testkit. This is a personal decision, not linked to or influenced by the data. Combining prior (external) information with data (using a likelihood function) is the domain of Bayesian statistics.

The challenge in this case is to examine whether or not the prior information is in accordance with the data. Test sensitivity and/or specificity may have been determined under conditions not compatible with a specific application of the test. For example, the test characteristics were determined in a temperate region using temperate breeds and the test is applied to tropical breeds in a tropical setting. Using the criteria outlined further it is possible to detect such a contradiction. As explained by Lesaffre et al. (2007) absence of proof is also here no proof of absence: not finding a contradiction between the data and the prior information does not guarantee that the final model yields the best possible estimates of test characteristics and true prevalence, only that there is no evidence of