

## CANINE LEISHMANIOSIS IN CROATIA – AN UPDATE

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### ABSTRACT

The present note reports the findings of the two parallel cross-sectional serological surveys for anti-leishmania antibodies in dogs. The first group consisted of 271 dogs from endemic area, and the second group consisted of 891 dogs living in the not endemic area.

In the first group 12/271 (4.42%) samples reacted positive with ELISA; besides ELISA, positive with IFAT and dipstick were 8/12 sera samples.

In the second group 19/872 samples reacted positive with ELISA (2.1 %). Along with ELISA, positive with IFAT were 6/19 sera samples and besides ELISA and IFAT, with dipstick reacted positive 5/19 sera samples.

### KEY WORDS:

Leishmaniosis, dog, Croatia, serology

### INTRODUCTION,

Leishmaniosis, due to *Leishmania infantum*, is a protozoan vector-borne disease and a serious public health problem. *L. infantum* causes the visceral and cutaneous leishmaniosis in humans and a systemic disease in the dog, considered the main reservoir of the infection (Gramiccia and Gradoni, 2005).

In the south littoral parts of Croatia, canine leishmaniosis had been recognized as a problem for the first time in the first part of 20th century. In Dalmatia region it was recognized as the re-emerging disease since 1997. The cumulative seropositivity of dogs in 2003 in the Split area was 15% (ŽIVIČNJAK et al., 2005); in the known enzootic area cumulative seropositivity of dogs recorded in 2005, 2006 and 2008 was 7.9%, 13.5% and 8.0% respectively (ŽIVIČNJAK et al., 2011). Leishmaniosis in clinically ill dogs from not endemic regions had been diagnosed in an average of 15 cases per year at the Faculty of Veterinary Medicine in Zagreb (unpublished data). Monitoring for anti-leishmania antibodies among dogs that were not suspected to be infected never had been performed in Croatia for an area outside the known endemic region.

### MATERIAL AND METHODS,

In Dalmatia was during April and May 2014 organised monitoring just for this purpose. In the city of Zagreb at the same time were tested the archive samples of processed dog sera that had been kept frozen (-20°C) for 1-2 months in refrigerators of few veterinary clinics from Zagreb. Belonging the first group, the dogs (mostly hunting dogs) were apparently healthy and lived in some of endemic areas of Dalmatia (Split and Sinj in Splitsko-dalmatinska county where stable foci of canine leishmaniosis had been reported previously, Knin in Šibensko-kninska county and Biograd n/m in Zadarska county, both with previously few unstable foci reported). The second group consisted of pet dogs not suspected by their veterinarians to have leishmaniosis, living in the not endemic area (mainly city of Zagreb).

A total of 1162 canine sera samples were examined. 271 dog sera samples originated from Dalmatia (Group 1) and 891 dog sera (Group 2) originated from continental area, mostly city of Zagreb.

The blood samples were tested for anti-leishmania antibodies with three serological tests. Two commercial serological tests were performed: indirect ELISA (INGENASA LEISHMANIA ING 15. LSH. K1) and the rK39 immunochromatographic dipstick test (Leishmaniasis Rapydtest® APACOR) along with modified *in house* indirect immunofluorescence method in serial dilutions (cut-off  $\leq 40^{-1}$ ). The antibody titre  $\geq 80^{-1}$  was regarded as positive. *In vitro* cultivation, antigen preparation and IFAT were performed as described by Mancianti et al. (1996), with modifications (Martinković and Marinculić, 2006). Commercial tests were performed according to the manufacturer's instructions. All sera were tested in duplicate and all were retested at least once.

### RESULTS,

In the Group 1 (dogs from endemic area) 12/271 (4.4 %) samples reacted positive with ELISA; besides ELISA, positive with IFAT and dipstick were 8/12 sera samples (66.6 %). IFAT antibody titre ranged from  $160^{-1}$ - $1280^{-1}$ .

In the Group 2 (dogs living in not endemic area) 19/872 sera samples reacted positive with ELISA (2.1 %); besides ELISA, positive with IFAT were 6/19 (31.6 %) samples (antibody titre range  $80^{-1}$ -  $640^{-1}$ ); besides ELISA and IFAT, with dipstick reacted positive 5/19 (26.3%) sera samples (all of them had IFAT antibody titre  $160^{-1}$  or higher).

## DISCUSSION,

Seroprevalence of canine leishmaniosis in endemic area virtually decreased compared to monitoring results in previous years. It could be attributed to the fact that the dog owners /especially hunters/ in this area often remove (euthanize or just relocate) clinically ill dogs and consequently reduce the source of infective meal for sand flies. Although it was not mandated by law, this attitude is supported by some veterinarians. Since the veterinarians and the most dog owners in the endemic area have been familiar with clinical signs of canine leishmaniosis demanding blood test on suspected dogs; along with preventive measures against sand fly bites, consequence of this may be an illusion that the disease is under the control. Hunters in Dalmatia usually own ten or even more hounds in a household; they swap, borrow, lease, sell and resell dogs, and it has been proven that a part of infected animals were sold or given away out of Dalmatia. Indeed, this particularly refers to apparently healthy seropositive dogs, being tested or not.

On the other hand, veterinarians and the owners in areas out of the endemic area suspect leishmaniosis seldom or with delay. Although many dogs from Croatia were bred and/or purchased from the endemic area (or just spent few summer days, weeks or months in Dalmatia), this fact has been rarely checked in anamnesis.

With ELISA test more sera samples reacted positive than with IFAT and therefore we applied the third test (dipstick). The latter two tests showed correlation for all but one of the sera obtained (belonging to Group 2). That particular sample reacted with the low antibody titre ( $80^{-1}$ ) in IFAT. Since all the samples that reacted positive for anti-leishmania antibodies with dipstick and IFAT were positive with ELISA it could be concluded that particular immunoenzymatic test was more sensitive, although nonspecific reactions have not been excluded. Those dogs should be monitored, retested and in vitro parasite isolation attempt has been planned for the following months.

## CONCLUSION

Early diagnosis of leishmaniosis in dogs is essential for surveillance and control programs. In the endemic area, measures consisting of education, serological monitoring, veterinary control of seropositive dogs and preventive measures against sand fly bites could support the decline of incidence.

On the other hand, in the not endemic areas, the disease is generally neglected, and awareness of this zoonosis should be elevated. Comprehensive interpretation of even slight biochemical abnormalities (thrombocytopenia or hyperproteinemia besides other regular findings, for example), and implementation of routine serological checkout of *Leishmania* antibodies with a high sensitivity test during routine laboratory evaluation should be reconsidered. Tests with high specificity we consider the best choice for ruling out infection in clinically ill dogs.

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