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RAPID STIMULATORY EFFECT OF LEISGUARD® ON THE CANINE INNATE IMMUNE SYSTEM

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Purpose of the work. *Leisguard® is a domperidone based oral suspension whose preventive efficacy against canine Leishmaniosis (CanL) has been demonstrated in several studies (Llinás J et al. 2011, Gómez-Ochoa et al. 2012a, Sabaté et al. 2012). Its repeated administration to healthy dogs during 30 consecutive days results in a stimulatory effect on the innate immune system, first defense barrier against the infection, being this the rationale for its clinical efficacy. More precisely, its active principle, domperidone, increases the percentage of activated phagocytic cells for at least one moth after treatment conclusion (Gómez-Ochoa et al. 2004, 2012b) leading to a significant increase of their anti-Leishmania activity in case of contact with the parasite (Gómez-Ochoa et al. 2013).*
Given that the above mentioned stimulatory effect is evidenced early after treatment initiation (Gómez-Ochoa et al. 2004), Leisguard® has been claimed to be a practical alternative to vaccination against CanL for preventing dogs living in non-endemic geographical areas occasionally travelling to endemic areas for short periods of time.
The objective of this study was to accurately determine how fast is the stimulatory effect of Leisguard® on the dog's innate immune system and for how long it remains active in healthy animals. This information in essential to establish an adequate prophylactic approach against CanL for travelling dogs.
Materials and used methods. *A total of 10 mixed breed healthy dogs of different sex (5 male and 5 female), age (3-12 years old), weight (8-31kg bw) were included in the study with the consent of their owners. All dogs were seronegative for anti-Leishmania antibodies (confirmed by a quantitative ELISA test both at the beginning and at the end of the study).*
The study had a duration of three months. During the first month (day 0 to 30) all dogs were orally administered 1ml/10kg/day of Leisguard® (equivalent to

0.5mg domperidone/kg/day). Blood samples were obtained at days 0, 1, 2, 3, 4, 5, 15, 30, 42, 58 and 92 in lithium heparin tubes and processed for the Nitroblue Tetrazolium test (NBT) following the instructions of the manufacturer (Sigma). The NBT is an assay based on the activation percentage of neutrophils in peripheral blood that has been proposed for the follow up of canine leishmaniosis owing to the narrow relationship between the molecules involved in the oxidative burst and the leishmanicidal activity of phagocytes (Gómez-Ochoa et al. 2010, Scarpona et al. 2010).

Mean percentage of activated neutrophils (NBT positive) at each day was compared to that of day 0 using the Repeated Measures ANOVA and the post-hoc Holm-Sidak method with an overall significance level of 0.05.

***Outcomes.** A low baseline percentage of activated neutrophils was detected in all dogs, prior to the first administration of Leisguard®, being this observation consistent with the results of previously published studies in healthy dogs (Gómez-Ochoa 2004, 2012b). From the first day of Leisguard® administration and during the 30-day administration period a progressive increase in the mean percentage of activated neutrophils was detected, with the highest percentages of activation being recorded on days 15 and 30. After treatment conclusion, mean percentage of activated neutrophils remained high up to day 58 and then decreased towards the baseline levels, which were reached on day 92. These results were again consistent with that reported in the literature for healthy dogs.*

The statistical analysis of the results evidenced significant differences in mean percentages of activated neutrophils vs. day 0 on day 2 ($p=0.03$), day 3 ($p=0.006$), days 4, 5, 15 and 31 ($p<0.001$), day 42 ($p=0.011$) and day 58 ($p=0.014$), therefore evidencing an extremely early stimulatory effect of Leisguard® on the dog's innate immune system that persists at least two month after treatment initiation.

***Conclusions.** Administration of Leisguard® to healthy dogs results in a statistically significant stimulatory effect of the dog's first defense barrier against Canine Leishmaniasis from the second day of treatment onwards. Consequently, dogs travelling to endemic areas can start treatment with Leisguard® very shortly before the trip or even upon arrival at their destination.*

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