TREATMENT WITH DIETARY NUCLEOTIDES AND ACTIVE HEXOSE CORRELATED COMPOUND REDUCES DISEASE PROGRESSION IN CLINICALLY HEALTHY LEISHMANIA-INFECTED DOGS

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ABSTRACT

Clinically healthy *Leishmania infantum*-infected dogs do not show clinical signs of leishmaniosis. Although treatment is not recommended in these cases, they should be managed in order to avoid disease progression and parasite transmission. Dietary nucleotides and active hexose correlated compound (AHCC) have demonstrated to modulate the immune response. A recent study in dogs with clinical leishmaniosis showed that 6-month oral administration of nucleotides plus AHCC achieves similar efficacy to allopurinol, without producing xanthinuria. **The present study was aimed at evaluating the effects of this combination to prevent disease progression in clinically healthy *Leishmania*-infected dogs.**

Forty-six dogs were included in this randomized, multicenter, double-blind, placebo-controlled trial. Dogs received once-daily oral administration of placebo or a supplement (**IMPROMUNE®**, Bioiberica S.A.U., Barcelona, Spain) containing nucleotides plus AHCC. At 0, 60, 180 and **365 days of treatment**, clinical signs using a validated score, and several biomarkers from blood, urine and bone marrow samples were evaluated.

A **significantly lower proportion of dogs (p=0.047) became sick in the supplement group** (3/20; 15%) compared to placebo (10/22; 45.5%). Compared to baseline, **ELISA-determined antibody titers were significantly reduced at all time points only with the supplement** (p<0.01). **Clinical score was significantly lower in the supplement** group after 180 days (p=0.014). No significant differences were observed for the other parameters. Both compounds were well tolerated.

In conclusion, oral administration of nucleotides plus AHCC for 365 days in clinically healthy *L. infantum*-infected dogs is safe and allows a significant reduction of anti-Leishmania antibodies and disease progression, hence exerting a preventive effect.

BACKGROUND AND OBJECTIVE

Canine leishmaniosis (CanL) caused by *Leishmania infantum* is a major global zoonosis potentially fatal to humans and dogs.[1] **Clinically healthy infected dogs** feature neither clinical signs nor clinicopathological abnormalities while having a confirmed infection. Although these patients are not sick and some of them may never develop clinical disease, they pose a therapeutic challenge. They may represent a risk of parasite transmission, especially if they progress to clinical leishmaniosis, and therefore need to be adequately managed.[2] However, the generalized use of leishmanicidal drugs on these dogs with subclinical infection is not advocated, in order to avoid the development of resistances.[1]

Orally administered **nucleotides** modulate the immune response, positively influencing lipid metabolism, immunity, and tissue growth, development and repair.[3] Active hexose correlated compound (**AHCC**), an alpha-glucan rich compound extracted from the mycelia of shiitake mushrooms (*Lentinula edodes*), is used in humans for its ability to stimulate the immune system.[4]

A recent randomized allopurinol-controlled trial in dogs with clinical leishmaniosis receiving an initial 28-day course of methylglucamine antimoniate (MGA) showed that oral treatment with nucleotides plus AHCC leads to similar efficacy to allopurinol, achieving better clinical results after six months and without producing xanthinuria.[5] **The objective of the present study was to evaluate the long-term effects of dietary nucleotides plus AHCC in clinically healthy CanL patients, and to assess whether this intervention could have a preventive effect protecting them from getting sick and start showing clinical signs.**

MATERIAL AND METHODS

Forty-six client-owned dogs with natural *L. infantum* infection were included in this randomized, multicenter, double-blind, placebo-controlled trial. All dog owners gave their written informed consent. Main inclusion criteria were: positive quantitative ELISA serology and positive cytology or PCR for *L. infantum*, while not presenting with clinical signs or clinicopathological abnormalities associated with CanL. Dogs were excluded if they had been vaccinated against CanL, or if they had received allopurinol three weeks prior to entering the study, or MGA, miltefosine, domperidone, ciclosporin, or glucocorticoids two months before the study start. The use of topical insecticides with a repellent effect against sand flies was allowed during the study.

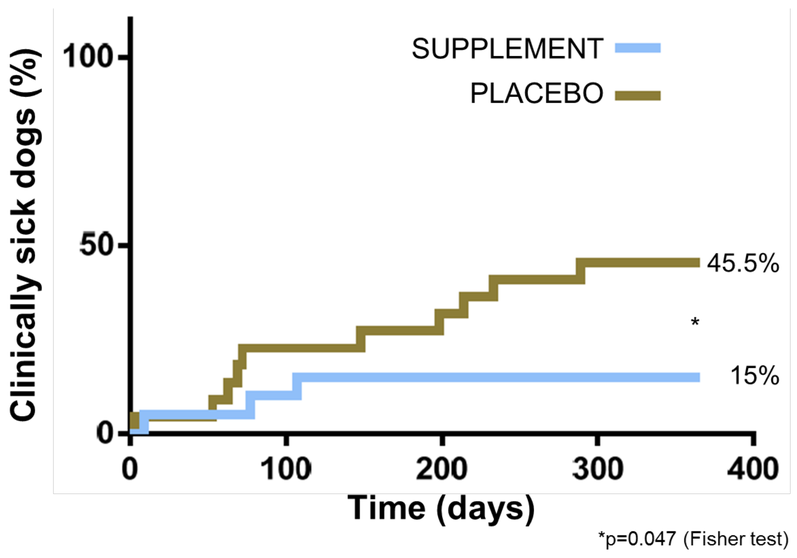
Enrolled dogs were randomly classified into **two groups** and received **once-daily oral administration of either a placebo or a dietary supplement** (**IMPROMUNE®**, Bioiberica S.A.U., Barcelona, Spain; 32 mg/kg nucleotides plus 17 mg/kg AHCC) for 365 days. **Disease progression –shift from being clinically healthy into sick patient– was monitored during the study in each group and used to assess the preventive effect of the supplement**. At 0, 60, 180 and 365 days of treatment, dogs were evaluated using a validated objective clinical score,[5] and a variety of analytes were measured from blood, urine (UPC, specific gravity and anomalies) and bone marrow (smears, qualitative PCR and RT-PCR) samples. Dogs with disease progression were excluded from the study and only their data until the last visit without having progressed to sick patients was used for the final data analyses.

Baseline differences were analyzed with a Student's t-test for quantitative variables and Fisher's exact test for categorical variables. Treatment effects were compared by analysis of covariance (ANCOVA) and changes over time were analyzed by repeated-measures analysis of variance (ANOVA). The level of statistical significance was set at 5%.

RESULTS

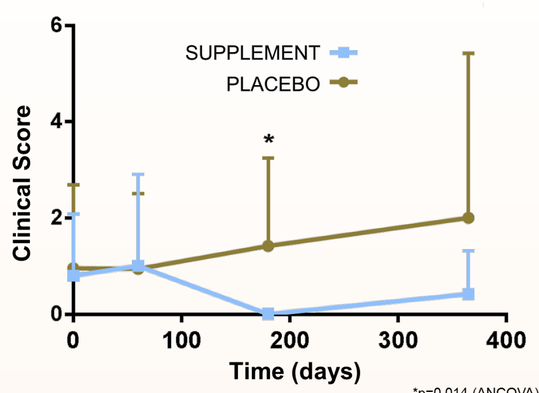
Initially, **the two groups were matched in terms of demographic and baseline characteristics** of the subjects. Dog breeds included in the supplement/placebo groups were as follows: Boxer (2/3), German Shepherd (2/2), American Staffordshire (2/0), Rottweiler (1/1), Siberian Husky (1/0), Doberman (2/2), Brittany Spaniel (1/0), English Bulldog (1/0), Labrador (1/0), crossed-breed (6/7), Dogue de Bordeaux (1/0), Cocker Spaniel (1/1), Majorca Ratter (0/1), Bullmastiff (0/1), Pit bull (0/2), Bodeguero Andaluz (0/1), French Bulldog (0/1), English Setter (0/1), Saint Bernard (0/1), Beagle (0/1). At baseline, there were no significant differences (p>0.05) between groups for any of the studied parameters. One dog in the supplement group and 3 in the placebo group were excluded due to reasons unrelated to disease progression.

- During the study, **3 dogs showed DISEASE PROGRESSION in the supplement group while 10 did in the placebo group (15% *vs* 45.5%; p=0.047)**

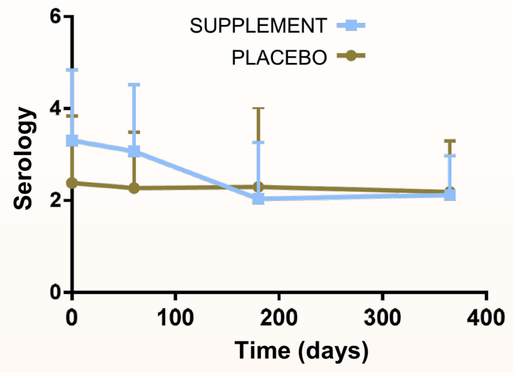


*p=0.047 (Fisher test)

- Due to the clinical worsening of dogs in the placebo group, after 180 days a significantly **lower CLINICAL SCORE was observed with the supplement (p=0.014)**.
- **ELISA serology was significantly reduced (p<0.01)** compared to baseline only in the **supplement group after 60, 180 and 365 days of treatment**.



*p=0.014 (ANCOVA)



- No significant differences were observed between groups or over time in blood CD4 and CD8 levels, and CD4/CD8 ratio, serum proteinograms, parasite burden, CBC and biochemistry, IRIS staging of chronic kidney disease, temperature or body weight.
- **Both the supplement and the placebo were well tolerated** and no side effects related to these compounds were reported in any patient.

DISCUSSION AND CONCLUSIONS

The oral administration of a dietary supplement containing nucleotides plus AHCC for 365 days in clinically healthy *L. infantum*-infected dogs is safe and leads to a significant reduction in the rate of disease progression and ELISA serology titers of antibodies against *Leishmania* infection. These findings point towards a possible preventive effect of such dietary supplement in CanL clinically healthy dogs, avoiding them to progress into sick patients.

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