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Clinical follow-up examination after treatment of canine leishmaniasis

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In 16 dogs, the diagnosis of canine leishmaniasis could be detected by direct microscopic identification, by determination of the antibody titre or by PCR method (peripheral blood/bone marrow). On the basis of the clinical and laboratory diagnostic results 9 cases of the cutaneous type and 7 dogs of the combined cutaneous-visceral type (+ mono- or polyarthritis, hepatopathy and/or renal insufficiency as well as occular manifestation) have been classified. Therapy was: *GLUCANTIME*® in 6 dogs, allopurinol in 3 dogs as single agent, combination-therapy *GLUCANTIME*® and allopurinol in 7 dogs. During *GLUCANTIME*®-treatment the following adverse reactions could be observed: general weakness, reduced food intake up to anorexia, vomiting and diarrhoea. Laboratory parameters showed sporadically leucopenia or pancreatitis. Adverse reactions during allopurinol therapy were: vomitus/diarrhoea or urine concrements. One dog with *GLUCANTIME*® therapy, 2 dogs with allopurinol as well as 2 dogs with combination therapy are clinically symptom-free at the moment (peripheral blood and bone marrow: PCR negative). The remaining 11 patients showed a good to very good improvement of the clinical symptoms. However, since the peripheral blood respectively the bone marrow continue to be PCR positive, relapses have to be expected in these dogs.

Keywords : Canine leishmaniasis, *GLUCANTIME*®, Allpurinol, Side effects, PCR

INTRODUCTION

On account of the international travelling with dogs, the occurrence of canine leishmaniasis (CaL) has to be expected in small animal practice within Germany or other primary non-endemic areas. [7, 9, 10, 12, 19, 22]. On one hand dogs accompany their owners on vacation trips to the mediterranean region, on the other hand the animals are imported from these countries to Germany by the vacationers or organisations for the protection of animals. The goal of these investigations was to check the treatment with *GLUCANTIME*® [8, 10, 11, 21, 22, 23, 24], allopurinol [4, 9, 13, 14, 23, 25, 26] or *GLUCANTIME*® and allopurinol in combination [22, 23, 25, 27] in 16 dogs naturally infected with leishmania infantum donovani clinically and with laboratory tests as well as to document the success/failure of the treatment by use of polymerase chainreaction (PCR) [1, 2, 16, 17, 20, 22].

MATERIALS AND METHODS

16 dogs, naturally infected with leishmania infantum donovani, were studied over a period of up to 48 months. Of these 9 male (56.3%) and 7 female (43.7%) dogs, 11 (68.8%) were mixed breed and 5 (31.2%) purebred dogs. In comparison to the total number of patients at our clinic (1990-1996: n= 10059: 56.4% male, 44.6% female, 20.9% mixed breed and 79.1% purebred dogs) it is conspicuous that more mixed breed dogs are affected. The cause of this coud be, that 10 dogs (62.5%) were imported mongrels from spain, whereas 4 pets accompanied their owner to Spain. In one dog the stay abroad was unknown and another one was born in Germany and had never been out of country, but the mother (affected with canine leishmaniasis) was imported from spain [7]. The clinical diagnosis was always confirmed

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Table 1 Signalement and clinical signs in 16 dogs with leishmaniasis and side effects to a treatment with *Glucantime*® (dog no. 1-6), allopurinol (dog no. 7-9), allopurinol & *Glucantime*® (dog no. 10-13) or *Glucantime*® & allopurinol (dog no. 14-16).

dog no.	sex	age (years)	clinical signs	laboratory findings before treatment	side effect of treatment	follow up (months)	outcome
1	male	8	alopecia, local dermatitis	no abnormalities	clinical no abnormalities mild proteinuria	26	no clinical signs, 1
2	female	2	alopecia, diffuse	marked hyperglobulinemia	lethargy, vomitus, diarrhoe	29	stable
3	male	8	lethargy, dermatitis with ulceration of the ear tip, seborrhoea	anemia, mild hyperglobulinemia	lethargy, reduced food intake leucopenia	48	no clinical signs, 2
4	male	5	facial desquamation, generalised dermatitis with ulceration of the ear tip, lymphadenopathy, lameness, weight loss	hyperglobulinemia	lethargy, vomitus	39	stable
5	male	2	vomitus, weakness, hepato- splenomegaly	renal failure, anemia, hyperglobulinemia, proteinuria	vomitus, pancreatitis	-	lost to follow up
6	female	3	seborrhoea, epistaxis, hyperkeratosis,	immunhemolytic anemia, hyperglobulinemia	leucopenia, melaena, vomitus	48	blindness,stable 1
7	male	10	seborrhoea, tracheal collapse	hyperglobulinemia	no abnormalities	12	no clinical signs, 1
8	female	4	seborrhoea ear tip, pressure point dermatitis	no abnormalities	no abnormalities	16	no clinical signs, 1
9	female	4	dermatitis	eosinophilia	no abnormalities	9	no clinical signs, 2
10	male	3	dermatitis, generalised lymphadenopathy	hyperglobulinemia	allopurinol: vomitus, diarrhoea *Glucantime*®: no side effect	39	no clinical signs, 3
11	male	5	pressure point dermatitis, face: alopecia local	hyperglobulinemia	Vomitus	9	no clinical signs, 2
12	female	2	alopecia, lameness, splenomegalie, generalised lymphadenopathy	hyperglobulinemia, anemia	vomitus, reduced food intake, leukopenia, allopurinol: urine with concrements	15	no clinical signs, 2
13	female	3	generalised dermatitis, alopecia, hepatopathy generalised lyphadenopathy	mild anemia, hyperglobulinemia hypoalbuminemia marked elevation of bile acids	lethargy	9	no clinical signs, 2
14	female	2	seborrhoea ear tip, generalised lymphadenopathy	hyperglobulinemia	lethargy	36	no clinical signs, 2
15	male	3	dermatitis with ulceration, weight loss, lameness	hyperglobulinemia	vomitus, pancreatitis	48	no clinical signs, 1
16	male	4	dermatitis, lameness	hyperglobulinemia	reduced food intake,sialoliths,	48	no clinical signs, 1

Further treatment: 1: no medication, 2: allopurinol, 3: *Glucantime*® weekly,

serologically (IFAT, ELISA) [1, 8, 10] and with cytological examination of a bone marrow aspiration. Peripheral blood and bone marrow were collected (before, during and after treatment) and precultured within RPMI culture medium, stored at room temperature, or with K_3-EDTA anticoagulated and frozen (−20℃) to performe PCR examination [20,22]. A full blood cell count (PCV, RBC, Hb, WBC, PLT, differential blood count) and measurements of total plasma-concentration of plasma proteins, albumin, globulins and fibrinogen, creatinine, urea, sodium, potassium, chloride, calcium, phosphate, glucose as well as the plasma-activity of alkaline phosphatase (AP), alanine aminotransferase (ALT), glutamat-dehydrogenase (GLDH), α-amylase, lipase and also urinanalysis were obtained from each dog before, during and after treatment. The therapy with the pentavalent antimonial n-methyl glucamin (*GLUCANTIME*®: day 1-2: 50 mg/kg bw and day 3-10: 100 mg/kg bw, intravenously diluted with 0.9% NaCl-solution, 14 days break and repetition of the course) was performed in 6 dogs and that with purine analogue allopurinol (2×daily 10 mg/kg bw) in 3 dogs. After a pretreatment period of 3 to 5 weeks in 4 dogs with allopurinol which showed an insufficient improvement, additionally *GLUCANTIME*® was used. The administration of allopurinol to 3 animals for 5 weeks up to 21 months was performed after an initial *GLUCANTIME*® course of treatment.

RESULTS

All dogs showed the typical skin symptoms (s. Table 1) reported in the literature [5, 9, 10, 19, 23]. In addition to these cutaneous forms of the disease 7 dogs were presented with visceral symptoms. In detail, the dogs no. 4, 12, 15 and 16 attract attention because of lameness (s. table 1) caused by mono- or polyarthritis [3, 28]. Further findings were renal failure [15,18] in dog no. 5, a case of hepatopathy [5] in dog no. 13 as well as ocular manifestation on dog no. 6 which lead to blindness.

The prominent clinical and laboratory findings before treatment are listed in table 1. It is remarkable, that specific leishmania antibody-titres of dogs with cutane symptoms ranges between 1:40 to 1:512, ab-titres from dogs with the cutan & visceral form on the other side are located between 1:512 to

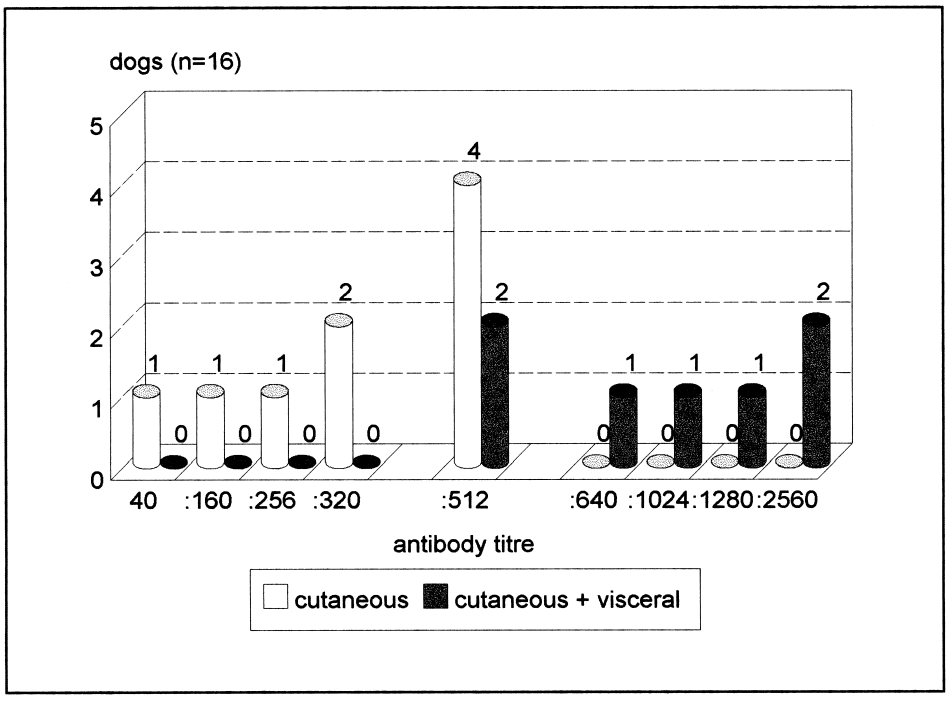

Fig. 1 Antibody titres in 16 dogs with leishmaniasis

1:2560 (s. Figure 1). The *GLUCANTIME*®-application lead to a good to very good reduction of clinical symptoms during the first 10 days of therapy. Side effects of *GLUCANTIME*® -application were (mostly day 4-5 of the treatment): vomitus (n=7), lethargy (n=7), reduced food intake up to anorexia (n=3), diarrhoea (n=1), melaena (n=1). With laboratory tests detected side effects were: leukopenia (n=3), increase of (-amylase- and lipase-activity in serum (n=2) and, only in dog no. 1, a mild proteinuria. Treatment-induced hepatopathy were not observed. In summary side effects of *GLUCANTIME*®-treatment were of a clinical nature in 75.0% (n=12) of the cases and only 37.5% (n=6) were evident on laboratory testing. With allopurinol as a single agent therapy, we succeded in reducing clinical symptoms considerable in three cases (dog no. 7, 8 and 9). But four dogs (dog 10 11, 12 and 13) pretreated with allopurinol showed no recovery and *GLUCANTIME*®-courses had to be added. With the exception of dog no. 3 (in this case the owner reported that vomitus and diarrhoea occured during therapy) and dog no. 12 (the urine contained small particles, no further examinations), no side effects were determined during the application of allopurinol.

One dog with a single *GLUCANTIME*®-treatment (n=7), 2 dogs with a single allopurinol treatment (n=3) and another 2 dogs with combined *GLUCANTIME*® and Allopuriol therapy (n=7) were PCR positive before and PCR negative after therapy.

DISCUSSION

In 16 dogs with naturally occuring leishmania infection the treatment with *GLUCANTIME*® and/or allopurinol was observed over a period of up to 48 months. *GLUCANTIME*® lead to a good to very good reduction of clinical symptoms in all treated cases, but with the exception of one case (dog no. 1), it could be proven by PCR, that the parasites survive in the bone marrow and a recurrence of clinical symptoms must be expected [12, 21, 22, 23].

As a reccurence of clinical symptoms can be expected after 6 months, PCR, which is a very specific and sensitive diagnostic method for initial detection of the disease [1, 2, 17, 20, 22], is the diagnostic method of choice for the detection of persistent infections after therapy [22]. Using PCR together with IgG1 and IgG2 [6] the success/failure of a therapy can be establihed. After clinical improvement allopurinol should be given long term. Monotherapy with allopurinol gives good results, if necessary *GLUCANTIME*® can be added to the treatment regime. The duration of treatment is not clear yet, it can vary from 5 weeks to 21 months. Even cases which had positive PCR results after *GLUCANTIME*® therapy can become PCR negative with additional allopurinol treatment (dogs no. 15, 16). Because of the appeareance of severe side effects [9, 11, 12] such as acute pancreatitis or leukopenia, *GLUCANTIME*®-application must be controlled by labaratory tests. After an initial *GLUCANTIME*® -application clinical remission can be maintained by additional treatment with allopurinol [23, 25, 26]. Side effects of this treatment such as vomitus and diarrhoea or urine concrements are very rare. In 2 cases with additional allopurinol application over period of 5 weeks /21 months, the dogs showed no clinical signs and the PCR results in the peripheral blood and bone marrow were negative. In 2 dogs treated with allopurinol as a single agent therapy, clinical signs disappeared and PCR results in the peripheral blood and bone marrow were negative.

This study suggests in accordance with the literature, that allopurinol is a very good mono- or additional therapy in canine leishmaniasis [9, 13, 14, 23, 25, 26, 27]. The benefit of longterm treatment with minimal side effects and in any cases negative PCR-results must be observed in future.

CONCLUSIONS

In cases of canine leishmaniasis, *GLUCANTIME*® therapy reduces clinical signs rapidly. As a reccurence of clinical symptoms can be expected after 6 months, PCR, which is a very specific and sensitive diagnostic method for initial detection of the disease, is the diagnostic method of choice for the detection of persistent infections after therapy.

The remission can be maintained with an additional allopurinol therapy. Even cases which had positive PCR results after *GLUCANTIME*® therapy can become PCR negative with additional allopurinol treatment (dogs no. 15, 16). After clinical improvement allopurinol should be given long term. The

duration of treatment is not clear yet, it can vary from 5 weeks to 21 months. Monotherapy with allopurinol gives good results, if necessary *GLUCANTIME*® can be added to the treatment regime.

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