The therapeutic effect of pour-on administered cypermethrin in *Psoroptes cuniculi* infestation in rabbits

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ABSTRACT

In this study, clinical and *in vivo* acaricidal effects of pour-on administered cypermethrin in rabbits with *Psoroptes cuniculi* were evaluated. Totally 24 rabbits were used in the study. Sixteen rabbits, naturally infested with *P. cuniculi* were randomly divided into two equal experimental groups and cypermethrin was pour-on administered 5 mg/kg (Group I) and 10 mg/kg (Group II) as a single dose. Also, 8 healthy animals (non-infested with *P. cuniculi*) exposed only saline were used for the control group. Psoroptic mange was diagnosed and monitored in the experimental groups by otoscopic and microscopic evaluations on Day 0 (pretreatment) and Days 1, 3, 7, 14, and 21 after the treatment. Clinical findings about psoroptic mange were scored, blood cypermethrin concentrations and haematological parameters were determined on the evaluation days. Six rabbits from Group I had *P. cuniculi* and a statistically important difference was determined between number of infested rabbits of Group I and Group II on Day 21 (p<0.05). Also, clinical scores of two groups were statistically different on Days 14 and 21 (p<0.05).

At the end of the study, only 10 mg/kg dose of cypermethrin could provide clinical recovery. No toxicological signs related to the therapy were detected in the all groups during the study. It was concluded that cypermethrin could be an effective, safe, and practical treatment alternative for *P. cuniculi* in rabbits. However, further studies should be performed for determination of the exact therapeutic doses.

Keywords: Cypermethrin, *Psoroptes cuniculi*, rabbits

RESUME

Efficacité thérapeutique de la cyperméthrine administrée en pour-on sur l’infestation par *Psoroptes cuniculi* chez le lapin

Dans cette étude, l’effet acaricide de la cyperméthrine administrée pour-on chez le lapin atteint de gale par *Psoroptes cuniculi* a été évaluée in vivo. Un total de 24 lapins a été utilisé dans l’étude. Seize lapins, naturellement infestés par *P. cuniculi* ont été répartis au hasard en deux groupes expérimentaux et la cyperméthrine a été administrée en pour-on à la dose de 5 mg / kg (groupe I) et 10 mg / kg (groupe II) en une seule dose. En outre, 8 animaux sains (non infestés par *P. cuniculi*) exposés seulement une solution saline ont été utilisés comme témoins. La gale psoroptique a été diagnostiquée et suivie dans les groupes expérimentaux par des évaluations otoscopiques et microscopiques au jour 0 (avant le traitement) et aux jours 1, 3, 7, 14, et 21 après le traitement. Les effets cliniques sur la gale psoroptique ont été mesurés, les concentrations en cyperméthrine dans le sang et les paramètres hématologiques ont été déterminés. Au jour 21, six lapins du groupe I avaient toujours des *P. cuniculi* et une différence significative sur le nombre de lapins infestés des groupes I et II a été observée (p <0.05). Par ailleurs, les scores cliniques des deux groupes étaient statistiquement différents aux jours 14 et 21 (p <0.05). A la fin de l’étude, seule la dose de 10 mg / kg de cyperméthrine a permis une récupération clinique. Aucun signe toxique n’a été détecté au cours de l’étude.

Mots clefs : Cyperméthrine, *Psoroptes cuniculi*, lapin

Introduction

*Psoroptes cuniculi* is an obligate, nonburrowing parasite causing severe dermatitis and otitis in domestic rabbits. The infestation has been found worldwide, with high prevalence. Clinical signs include intense pruritus and head shaking with subsequent serum exudation and crusting on the pinnae, which are painful, in rabbits. Self-excoriation may lead to secondary bacterial infections [2].

Pyrethroids, ivermectin, moxidectin, selamectin, doramectin and eprinomectin, have been reported to be effective against *P. cuniculi* infestation in rabbits.[4, 6, 7, 11, 12] Cypermethrin is a widely used synthetic pyrethroid and its protective and therapeutic effects have been demonstrated against a variety of ectoparasites in dairy cattle, beef cattle, sheep, and horse as *in vitro* and *in vivo* topical application [3, 9, 12, 13]. However, there is no report regarding *in vivo* efficacy of cypermethrin against *P. cuniculi* in rabbits.

The aim of the present study was to investigate the clinical and *in vivo* acaricidal effects of pour-on administered cypermethrin in rabbits with *P. cuniculi*. In addition, relationships among clinical recovery, haematological parameters, adverse effects and blood cypermethrin levels during the study were evaluated.

Materials and Methods

ANIMALS

The study was performed at the Medical and Surgical Research Centre of Faculty of Medicine, Ondokuz Mayis University. Twenty-four adult New Zealand white rabbits (13 females and 11 males, 2-4.2 kg) were used. Sixteen rabbits,
which were naturally infested with \textit{P. cuniculi}, were randomly divided into two equal experimental groups. In addition, eight healthy animals (non-infested with \textit{P. cuniculi}) were used for the control group. Each group was housed in a separate room and each animal was individually kept in separated wire cages (0.5 m²). The animal rooms were maintained at 22°C ± 2°C, 60% ± 5% relative humidity, and 12/12 h light/dark cycle. Commercial rabbit pellets and water were available \textit{ad libitum}. The experimental protocol was approved by the Experimental Animal Studies Ethics Committee of Ondokuz Mayis University (No: HADYEK/83).

**EFFECT OF CYPERMETHRIN IN \textit{P. CUNICULI}**

Before the treatment (Day 0), all rabbits in the experimental groups had alive \textit{P. cuniculi} mites (Figure 1). Six rabbits infested with \textit{P. cuniculi} in Group I on Day 7 and this count did not change until Day 21. Infested rabbit number and limit of quantification (LOQ) values were calculated according the previous studies [1, 18].

**DIAGNOSIS OF PSOROPTIC MANGE**

At the pre-treatment assessment (Day 0), 16 rabbits were diagnosed with Psoroptic mange by direct observation of viable mites in all animals with an otoscope and by microscopic examination of scab materials under low power examination (4x). Each scab material was collected over an area at four different sites. The sample obtained sites were selected based on individual clinical signs. Scab materials were collected on each examination day. Microscopic examinations were performed by a parasitologist unaware of the groups.

**CLINICAL EXAMINATIONS**

Both ears of rabbits were examined clinically with visual and otoscopic inspections and evaluated for the presence of clinical signs consistent with Psoroptic mange such as erythema, scaling-crusting, papules, alopecia, and excoriations. The extent of the lesions was scored objectively as soon as possible (0: ears that appeared normal; 1: lesions confined to the ear canal; 2: lesions within the lower third of the auricle; 3: lesions within the lower two-thirds of the auricle; 4: lesions that extended over greater than two-thirds of the auricle) [10].

**TREATMENT**

Cypermethrin (Ektovet, VETAS A.S., 2.5% pour-on solution, Turkey) was administered for a single dose, 5 mg/kg (Group I) and 10 mg/kg (Group II) directly to the skin on the dorsal side of the animals. The application area was not covered, but any grooming and ingestion behaviour was not observed during the study. In order to create same stress conditions with experimental groups, only saline solution was applied via the same route in control animals which were not treated with cypermethrin.

**FOLLOW-UP EXAMINATIONS**

Otoscopic and microscopic examinations were performed and besides the clinical observations, whether the presence of alive mites on rabbits were evaluated on Day 0 (pretreatment) and Days 1, 3, 7, 14 and 21 after the treatment. Furthermore, blood cypermethrin concentrations and haematological parameters were determined on the same days.

**DATA EVALUATION**

Statistical differences in the haematological parameters were determined by one-way ANOVA, followed by Tukey’s and Dunnett tests in certain experiment times. In addition, clinical scores, number of rabbits that had mites and blood cypermethrin concentrations were evaluated by Mann Whitney U Test or Wilcoxon Test. A two-way ANOVA for repeated measures (group x time), followed by Tukey’s test were used for evaluation of time-depended changes in the obtained data. All data were expressed as mean ± standard deviation (SD). Differences were considered as significant when \( P \) values were less than 0.05.

**Results**

**EVALUATION OF \textit{P. CUNICULI} INFESTATIONS**

Before the treatment (Day 0), all rabbits in the experimental groups had alive \textit{P. cuniculi} mites (Figure 1). Six rabbits infested with \textit{P. cuniculi} in Group I on Day 7 and this count did not change until Day 21. Infested rabbit number was decreased during the experiment in Group II and any mite did not be detected on Day 21. A statistically important difference was determined between two groups at the end of the study (\( P < 0.05 \)).
CLINICAL EXAMINATIONS

Clinical scores of the groups are shown in Figure 2. Clinical scores of Group II were determined lower than Group I from Day 3 and a statistical difference was observed between two groups on Days 14 and 21 (p<0.05). All rabbits in Group II clinically recovered at the end of the study (Figure 3). However, the ear or skin lesions did not recover completely, and enough therapeutic effect for *P. cuniculi* was not observed in Group I (six animals had alive mites and clinical score was still high on Day 21). No toxicological sign about the therapy was detected in the all groups during the study.

BLOOD CYPERMETHRIN CONCENTRATION

Blood cypermethrin concentrations of the experimental groups are shown in Figure 4. Blood cypermethrin concentration of Group II was determined higher than Group I in all experiment times. Cypermethrin percentage of the used commercial product was measured 2.44% and evaluated as compliant to the tolerance limits (2.125-2.875) [17]. The mean recovery percentage, LOD and LOQ values were determined as 78.65±17.22%, 10.64 ng/ml and 35.47 ng/ml, respectively.
EFFECT OF CYPERMETHRIN IN P. CUNICULI

HAEMATOLOGICAL PARAMETERS

RBC value decreased on Days 7, 14 and 21 and Hb, Ht, MCV, MCH and MCHC values decreased in almost all examinations in Group I (p<0.05). Also, WBC value of Group I was detected increased compared with the control group in all examination times. In Group II, only WBC value was determined statistically different from the control group until Day 3 (p<0.05) and another failure was not observed during the study (data not shown).

Discussion

Ear mange in rabbits caused by P. cuniculi is very important with respect to general animal health and hygiene, and economic concerns [15]. P. cuniculi has various life stages, egg, larva, nymph and adult mite with a 21 day life cycle, depending on environmental conditions. Therefore, effects of cypermethrin were evaluated during 21 days in the present study.

Treatment of ear mange in rabbits is done by using a number of different preparations [15]. Several pour-on formulations of organophosphates and ivermectin as well as synthetic pyrethropoids have been used for P. cuniculi control, but all failed to eliminate the mite fully [8].

Cypermethrin is a synthetic pyrethroid that was first employed as an ectoparasiticide in 1978 by Halls and has been widely used since then for control of various harmful organisms [9]. Cypermethrin is used for scabies in farm animals at a general dose of 5 mg/kg. Pap et al. [11] and Coles and Staffort [3] reported that cypermethrin is effective against rabbit ear mite as P. cuniculi in vitro. But, no report could be found about in vivo therapy of P. cuniculi with cypermethrin in rabbit. In this study, 5 (the common dose for scabies) and 10 mg/kg doses of cypermethrin were pour-on administered for treatment of P. cuniculi infestation and its clinical and haematological effects were evaluated during 21 days in rabbits.

After cypermethrin administration, decreased clinical scores that were indicators of the Psoroptic mange, observed in both experimental groups, However, this effect was predominant in Group II and continued until Day 21. Clinical scores of Group I were higher than Group II from Day 3 to end of the study, and enough therapeutic effect of cypermethrin therapy for P. cuniculi did not observed in Group I. P. cuniculi infested rabbit numbers were decreased by 10 mg/kg cypermethrin during the study. However, infested rabbit count was always high and 6 out of 8 rabbits had alive P. cuniculi on Day 21 in Group I.

Jana et al. [5] detected that RBC and Hb levels decreased in P. cuniculi infested rabbit. Similarly, low RBC, Hb, Ht values were observed especially in Group I until Day 21 in this study. In addition, other haematological changes, such as leucocytosis, observed especially in Group I and these findings probably depended on severe scabies infestation and inadequate recovery process. However, these significantly abnormal haematological changes were not detected in Group II and this finding can be indicated the efficacy of 10 mg/kg dose for P. cuniculi infestation.

Gas chromatography is a specific and a very sensitive analytical method for pyrethroid analysis in various matrixes [18]. In the present study, blood cypermethrin concentration was determined by gas chromatography for detection of possible relationships among efficacy and adverse effects. Naturally, mean blood concentrations of Group II was higher than Group I during the experiment and efficacy of 10 mg/kg dose for mites can be depended to higher blood cypermethrin concentration.

Acute and short term dermal toxicity of cypermethrin is of a low order. However, sedation, ataxia, tremors and convulsions can be observed in after oral and dermal administration, especially in higher doses [16]. In the present study, any important adverse effect was not observed in the measured blood cypermethrin levels.

In this study, the efficiency and safety of cypermethrin was evaluated using a control group consisted of non-infested animals which exposed only saline solution and the main disadvantage of the study was lack of a positive control group (infested animals without treatment). This study was carried out in naturally infested animals which were housed an research centre of experimental animals and we could not use a positive control group due to risk factors (lethal effects of P. cuniculi infestation, such as considerable weight loss, vestibular dysfunction, and meningitis, which is frequently lethal when complicated by the secondary bacterial pathogens). Although this disadvantage, the present study showed that pour-on administration of 10 mg/kg dose of cypermethrin was effective for P. cuniculi in...
rabbits. However, 5 mg/kg dose was not sufficiently effective. Any important adverse signs were not detected in both experimental groups during the study. On the other hand, haematological abnormalities, which were decreased RBC, Hb, Ht and increased WBC were observed generally in Group I and a relationship was not observed between blood cypermethrin concentrations and these failures.

Consequently, cypermethrin can be an effective, safe and practical treatment alternative for *P. cuniculi* in rabbits. However, further long term studies with positive control groups should be performed for determination of the exact therapeutic doses.

References


