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Comparison of the Effects of Local and Uncontrolled Levamisole Preparations on Mice Naturally Infected with *Aspiculuris tetraptera*

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SUMMARY: This study was performed to compare effectiveness of two levamisole preparations prepared in two different countries (Iran and Turkey) in mice naturally infected with *Aspiculuris tetraptera*. For this purpose, natural infection was diagnosed using the cellophane tape method on the perianal region and centrifugal flotation technique on the feces of mice obtained from the experimental Animal Unit of the Faculty of Medicine, University of Yüzüncü Yıl, Van. Mice naturally infected with *A. tetraptera* were then divided in three groups. Animals in Group 1 (8 animals) received levamisole prepared in Iran, animals in Group 2 (8 animals) received levamisole prepared in Turkey and animals in Group 3 (6 animals) were used as untreated controls. Both levamisole preparations were used in a 10 mg/kg dose. After drug administrations, stool samples of the animals in all groups were examined for seven days. On the eighth day, the animals were humanely destroyed using inhalation anesthesia. After euthanasia, parasites in the intestine were also counted. As a result; levamisole coming through uncontrolled border trade from Iran was 69.3% effective against *A. tetraptera* and the levamisole prepared in Turkey was 91.7% effective in naturally infected mice. Results obtained from this study compared statistically and the differences were found to be significant ($p<0.001$).

Key words: Levamisole, *A. tetraptera*, Mouse, anthelmintic effect

Aspiculuris tetraptera ile Doğal Enfekte Farelerde Yerli ve Kontrolsuz Levamizol'un Karşılaştırmalı Etkisi

ÖZET: Bu çalışma, *Aspiculuris tetraptera* ile doğal enfekte farelerde iki farklı ülkede (İran ve Türkiye) üretilen levamizol (Levamisole) preparatlarının etkinliklerini karşılaştırmak amacıyla yapıldı. Bu amaçla, Yüzüncü Yıl Üniversitesi Tıp Fakültesi Deney Hayvanları ünitesinden temin edilen farelerin santrifüj flotasyon yöntemi ve selofan bant tekniği ile parazitolojik muayeneleri yapıldı. Yukarıdaki muayenelerle *A.tetraptera* ile enfekte oldukları tespit edilen fareler 3 gruba ayrıldı. Birinci gruptaki hayvanlara (8 fare) İran orijinli levamizol, ikinci gruptaki hayvanlara (8 fare) Türkiye orijinli levamizol uygulanırken, üçüncü grup fareler (6 fare) tedavi edilmeyen grup olarak kullanıldı. Her iki levamizol preparasyonu da 10 mg/kg dozunda verildi. İlaç uygulamasından sonra bütün gruplardaki farelerin dışkı örnekleri 7 gün süre ile alınarak incelendi. Sekizinci gün bütün fareler insancıl bir şekilde inhalasyon anestezisi kullanılarak öldürüldü. Ötenazi sonrası bağırsaklardaki parazit sayımları da yapıldı. Sonuç olarak, kontrol edilemeyen sınır ticareti ile İran'dan getirilen levamizol %69,3 oranında, Türkiye'de üretilen levamizol ise %91,7 oranında doğal olarak *A. tetraptera* ile enfekte farelerde etkili oldukları belirlendi. Elde edilen bulgular istatistiksel olarak karşılaştırıldı ve farklılık önemli ($P<0.001$) bulundu.

Anahtar Sözcükler: Levamizol, *A. tetraptera*, Fare, Anthelmintik etki

INTRODUCTION

One of the most important production loss in the farm animals is helmint infections. It is well known that helmint infections ratio is nearly 100% in this country which cause high economic losses (15). To control such infections, several different anthelmintic drugs have been used. For this purpose, moxidectin, ivermectin, doramectin netobimin and benzimidazole groups have been used (6, 7, 10, 11, 16). However, using these drugs at randomly in the treatment of helmint infections cause anthelmintic resistance in the helmint (5, 14, 20). One of

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the drug used against to helmint infection in domestic animals is levamisole, which is used at 7.5-10 mg/kg dose. Its effect occurs through paralysis on the parasite, by damaging fumarat reductase enzyme effectiveness. Levamisole is also known to stimulate immune system of the host (1, 10, 12, 13, 17).

There are several drugs coming through uncontrollable border trade from neighbouring countries to Turkey. These drugs have been used in the treatment of several diseases without licence and veterinary prescription (21). Therefore, in the present study, the effectiveness of two same drugs, one produced in Iran brought to Turkey by uncontrollable and the other produced in Turkey, were aimed to investigate against *A. tetraptera* in naturally infected mice.

MATERIALS AND METHODS

In this experimental study, 22 Swiss albino mice which were obtained from animal house, in the Faculty of Medicine, University of Yüzüncü Yıl, Van were used. To detect naturally infected mice a hundred stool samples were examined. Infected animals with *A. tetraptera* were determined by the technique of centrifugal flotation in saturated zinc sulphate solution and cellophone tape method on the perianal region.

Twenty two infected mice determined by stool sampling were divided into three groups by randomly selection. Each treatment groups had 8 animals and a control group contained 6 animals which female and male animal numbers were equal.

Mice in Group 1 received levamisole (originated from Iran named levamisol) at 10 mg/kg in single dose and mice in Group 2 received levamisole (originated from Turkey, Nilverm fort R, SanofiDif) at the same dose orally. Mice in Group 3 (control) received same amount of serum physiologic orally. The animals were housed at room temperature (20±2 °C) in standard cages with food (obtained from Van animal feed factory, Van-Turkey) and water ad libitum, in rooms lightened in a rhythm of 12 hours light, 12 hours dark and about 45% relative humidity.

The stool samples from mice were examined one day before the treatment, on the day of the treatment and daily for 7 days after treatment using centrifugal flotation technique in saturated zinc sulphate. The necropsy was humanely applied (by deep ether anesthesia) to both treatment groups and control group on the eighth day after the treatment. Gastro-intestinal tract was opened and washed with serum physiologic. The contents were examined under a stereomicroscope to amount and identify it any parasite present. The percentage effectiveness of the drugs were calculated by the formula given below (8, 9, 18).

$$\text{Efficacy (\%)} = \frac{\left(\begin{array}{c}\text{Geometric mean number of}\\ \text{A. tetraptera in control group}\end{array}\right) - \left(\begin{array}{c}\text{Geometric mean number of}\\ \text{A. tetraptera in treated group}\end{array}\right)}{\text{Geometric mean number of A. tetraptera in control group}} \times 100$$

Data from treated and control mice were calculated statistically in order to evaluate their significance using variance analysis test and importance test between the results obtained as percentage.

RESULTS

In the present study, the number of infected mice started to decrease on the second day of the treatment in both treatment groups (Group 1 and Group 2). In the necropsy; severe parasite invasion seen in the control group mice. Furthermore, all mice in Group 1, had the parasite at necropsy and a total of 152 parasite counted. On the other hand, mice in Group 2, three mice had no parasite, and the other 5 had a total of 70 parasites at necropsy. In the control group 442 parasites were counted When percent efficacy calculated; it was 69.3% in Group 1 and 91.7% in Group 2. The results were given in table 1 in detailed.

The differences between the ratio of percent efficacy between groups were statistically important ($p<0.001$). In addition, side effets in the animals due to the drugs were not observed in both groups.

Table 1. The efficacy of two levamisole preparations (originated from Iran and Turkey) against naturally infected mice with *A. tetraptera*

Groups	Number of mice with parasites at necropsy	Parasites counts recovered at necropsy (days 8)					Efficacy %
		Total	Min-max	Geo-mean	SE	SEM	
Group 1 n:8	8	152	4-32	16.2088	9.82344	3.47311	69.3
Group 2 n:8	5	70	1-23	4.3593	9.74588	3.44569	91.7
Group 3 (Control) n:6	6	442	18-175	52.718	65.30748	26.66167	

SE: Standard Deviation; **SEM**: Standard Error of Mean

DISCUSSION

In some studies, 90-100% effectiveness of levamisole against gastrointestinal nematodes in domestic animals have been repor-ted (5, 12,13). In the present study, two levamisole preparates were used and their effectiveness investigated in mice naturally infected with *A.tetraptera*. According to our results, levamisole originated from Iran had 69.3% and the other levamisole originated from Turkey had 91.7% effectiveness against *A. tetraptera* in naturally infected mice. Despite number of eggs seen with visual estimation did not change during this study in the control group, number of eggs obtained from Group 1 was observed to be higher compared with Group 2. The levamisole originated from Iran is coming through uncontrollable border trade. Their prescription informations may not be true. Because it is a material of an unofficially trade it can

not be controlled in terms of effective agents amount in a bolus.

Normally (In fact), their effectiveness shouldn't be different. In some studies several factors reported to cause reduction on the effectiveness of antelmentics such as, expired last usage date, damaged packing, expose to direct sun light, heat and humidity, keeping in unsuitable places (3, 5, 10, 17). In a study about illegal drugs carried out in Van province (21) was reported that, 60% of these uncontrolled (illegal) drugs last usage date expired, most of them taken out from their packing and exposed to direct sun light and sold without any permission in bazaars. Low effectiveness of the Iran originated levamisole determined in the present study could be partly due to several reasons mentioned above (above reasons). The other reason was that the development of resistance which occurs as a result of long term usage in an area especially at low doses, having low effective level and damaged structure. This was a small possibility in the present study. Because, if so, it should happened to the other drug originated from Turkey. On the other hand, such results have been reported by several researchers with regard to resistance development (2, 5, 14, 20). Çırak et al (4) investigated anthelmentic resistance, and found that cyathostomin nematods developed resistance against to benzimidazole group drugs. Therefore, in this region extensive and serious studies shoul be performed with regard to resistance against anthelmentics.

As a result, levamisole coming through unofficially border trade had statistically lower ($p<0.001$) effect against *A. tetraptera* in mice after natural infection compared to licenced and controlled levamisole originated from Turkey. We suggest that precautions against uncontrollable border trade should also be taken because of the drugs having low effect rate should not be used in the region for a long time in order to prevent developing of resistance (because of the development of resistance).

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