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Effective treatment regimen for control and eradication of oxyurids in laboratory rodents

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Abstract

Pinworm infestation is more common in most conventional colonies of laboratory rodents. They usually do not exhibit any clinical signs and no adverse effects have been reported on reproduction in infested animals, although, there is an inverse relationship between bodyweight and degree of infestation in newly weaned rodents. It has been reported that oxyurid infestations influence experimental results and also interfere with research goals in number of ways. The prevalence of pinworms in rodent population depends on many factors, including environmental load, gender, age, strain, and immune status. Unlike many common viral diseases of laboratory mice, pinworm infestations can be treated. A rederivation program is highly recommended if other pathogens are present in the colony. Present study was carried out to evaluate the effective treatment regimen for oxyurid infestation. Rodent colonies were screened for adult oxyurid worms or eggs by cellophane tape or flotation method. This screening revealed the presence of pinworm in mouse and rat colonies. Treatment regimen included administration of combinations of fenbendazole and praziquantel for group I at the rate of 150 mg/kg and 50 mg/kg respectively for 24 days through drinking water. Piperazine and ivermectin was administered for group II at the rate of 10.8 mg/mL for 14 days and 0.01mg/mL for next 14 days respectively through drinking water. No oxyurids were found in the colony after 28 days of treatment in group II. It was concluded based on the treatment regimen that piperazine and ivermectin combination was more effective in complete eradication of oxyurids in rodents.

Key words: Oxyurids, rodents, ivermectin, piperazine

Introduction

Throughout the history, scientists have been solving medical problems, developing new techniques and treatment regimens by using animal models in biomedical research. The use of laboratory rodents as a research animal has resulted in many scientific advancements. Much of our early understanding was consequent from studying the mouse and its use continues to be an important part of various research endeavors such as aging, embryology, cancer biology, pharmacological and toxicological testing and infectious disease research. Transgenic and knockout mice are being used as important tools for investigating the relationship of genetic make-up to disease status as well as elucidating pathways of normal mammalian development. Virtually every major medical advance in both humans and animals has been achieved through animal models. Most of the conventional laboratory rodent colonies are commonly infested with oxyurids namely, *Syphacia obvelata, Syphasia muris* and

Apicularis tetraptera (Habermann and Williams, 1958; Stahl, 1961; Flynn, 1973). Since, clinical signs are rare in most infested animals, body condition or general health does not generally preclude these animals from being used in research. Pinworm infestation may have more subtle effects, generally affecting the nature of the immune response that may render animals unsuitable for use. Mice which are infested with pinworms had a greater incidence of autoimmune diseases, hematopoietic and lymphopoietic diseases and an increased allergic response to a dietary antigen whereas nude mice had increased prevalence of lymphoma (Charles River, technical sheet). Eradication of pinworm infestation is extremely difficult, especially in large rodent breeding colonies. For effective control, breeding colony should be started with parasite-free parents that are derived either through caesarian or embryo transfer or appropriate treatment regimen to obtain parasite-free offspring.

Several techniques are being used to diagnose pinworm infestations in the laboratory rodents. These include the perianal tape test (Eguiluz *et al.,* 2001), anal swab technique (Goncalves *et al*. 1998), fecal flotation and necropsy with direct examination of the colon or cecum contents by using a filtration procedure (West *et al*. 1992; Klement *et al*. 1996). In rats, the perianal tape test had 88% sensitivity for *S. muris* (Huerkamp, 1993).

A number of strategies for pinworm eradication have been used in the laboratory rodent colonies. In 1950s, several compounds were noted to have an anthelmintics effect and were used with varying degrees of success which includes pyrvinium pamoate, fenbendazole, ivermectin, doramectin, moxidectin, piperazine, thiabendazole and mebendazole (Blair *et al*. 1968; Taffs, 1976; Battle *et al*. 1987; Klement *et al*. 1996; Zenner, 1998; Huerkamp *et al*. 2000; Oge *et al*. 2000; Pritchett, 2002). These compounds were not as effective as GABA-agonistic piperazine compounds that are being widely used and in combination with ivermectin or benzimidazole (Zenner, 1998; Taffs, 1976; Lipman *et al*. 1994; Owen and Turton, 1979; Unay and Davis, 1980; Taylor, 1992; Wagner, 1970; Kantee, 1973; Reiss *et al*. 1987 and Martin, 1997).

The most popular agents in use today for pinworm eradication are piperazine and avermectins. Piperazine is an organic compound, formed as a co-product in the ammoniation of 1, 2-dichloroethane or ethanolamine and it generally causes paralysis mediated by its agonist effects upon the inhibitory GABA receptor of helminths whereas, the avermectins are macrocyclic lactones produced by the actinomycete, *Streptomyces avermitilis* and their mode of action is to paralyze parasites by increasing muscle Cl^- permeability through a glutamate-gated ion channel (Martin, 1997). The avermectins are represented mainly by ivermectin.

The present study describes an effective treatment regime to control oxyurids infestation in laboratory rodent colonies by comparing the combinations of fenbendazole plus praziquantel and piperazine plus ivermectin administered orally through drinking water.

Materials and Methods

Animals

The present study was conducted in a laboratory rodent colony, bred and maintained at the Central Animal Facility, Indian Institute of Science, Bangalore, Karnataka. The animal facility is registered with the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments in Animals) for animal experimentation. The colony consists of several strains of genetically modified and wild type mice and also two strains of rats. All the animals were maintained under standard husbandry conditions with temperature ranging from 22 ± 2^oC and $55 \pm 5\%$ relative humidity with light and dark schedule of 12:12 hrs. Purified water (Aquaguard) was given *ad libitum.*

Sample collection:

Fresh faecal samples from mice and rats which were collected as a part of the routine health monitoring programme were used in this study.

Diagnostic methods:

1. ***Direct examination/ Floatation method:***

Fresh and wet preparations of fecal pellets were observed under microscope for endoparasites followed by separation of ova by flotation method using saturated sodium chloride solution. Approximately 2- 3 fecal pellets were triturated and 5 ml of flotation fluid was added into a container up to the brim. The container was allowed to stand for 10 minutes and placed the clean nongreasy slide over the mouth of the container for 10 minutes. The slide was lifted and then inverted quickly on the right side for observation under low power objective (10x).

2. ***Cellophane tape impression test:***

A strip of transparent cellophane tape was pressed against the area of pelt at perianal region of randomly selected animals and then placed sticky side down on a clean non greasy microscopic slide and the entire slide was examined thoroughly under low power objective of compound microscope (Graham, 1941; Jacobs, 1942) to detect the presence of ova of *Syphacia spp* and *Aspiculuris tetraptera*

Treatment regime:

Treatment regimen included administration of combinations of fenbendazole and praziquantel (FENTAS PLUS, oral suspension, INTAS pharm, Ahmedabad) for treatment group I at the rate of 150 mg/kg and 50 mg/kg respectively for 24 days. The piperazine (Piperazine hydrate, Brihans Laboratories, Bangalore) was administered orally through drinking water at the rate of 10.8 mg/ml from day 0 to day 14. Subsequently, ivermectin (Neomec, ivermectin oral suspension, INTAS pharma, Ahmedabad) at the rate of 0.01 mg/ml through drinking water from day 15 to 28 for treatment group II.

Results

This study was conducted to determine the efficacy of combination therapy on oxyurids. The treatment regime used and the number of fecal samples screened in this study is provided in table 1. Current study revealed the presence of pinworms in the rodent colony and the results are provided in table 2. Perianal impression revealed the presence of oxyurid eggs (fig.1). It was observed that combination of piperazine and ivermectin was highly effective over the combination of fenbendazole and praziquantel (table 3). Few eggs were observed in treatment group 1 even after 21 days of treatment and no eggs were observed in treatment group 2.

Discussion

However, the results of various studies on the efficacy of these compounds vary in their doses, duration and route of administration. MacArthur (1978) has reported that continuous medication using 0.1% thiabendazole in th diet for up to 24 days was effective in eliminating both *S obvelata* and *A. tetraptera*. In our study, 14 days treatmer regime of piperazine with the dose 10.8 mg/ml along wit 14 days treatment of ivermectin at the dose rate of 0.01mg ml eliminated oxyurid infestation from the colony. Afte the 28th day of treatment, no adult pinworms and eggs wer found. Combination therapies of piperazine and ivermecti in drinking water were found to be effective in eliminatin oxyurid infection in mice and rat colonies (Kathleen an Johnston, 2002). The gold standard for eradication of pi worms is still rederivation of rodents via hysterectom and caesarian section or embryo transfer (Morell, 1999) However, in many situations, rederivation is not practical. I view of this, appropriate treatment is indicated to eradicat the pinworm infestation. Fenbendazole is recommended ove ivermectin because no documented evidence to show it interference with research, its wide margin of safety and i ovicidal, larvicidal and adulticidal effects.

Table 1: Treatment regime in rodents

Type of stock	Number of animals		Treatment Group I	Treatment Group II
	Males	Females		
Mice	40	40	Fenbendazole (150 mg/kg) and Praziquantel (50 mg/kg) 24 days	Piperazine (10.8mg/ml) 0-14 days and Ivermectin (0.01 mg/ml) 14-28 days
Rat	40	40		

Table 2: Results of Tape impression test before treatment

Type of stock	Treatment Group I		Treatment Group II	
	Males (n=40)	Females (n=40)	Males(n=40)	Females(n=40)
Mice	33	31	30	31
Rat	29	30	28	30

Table 3: Results of Tape impression test after treatment

Type of stock	Treatment Group I		Treatment Group II	
	Males (n=40)	Females (n=40)	Males(n=40)	Females(n=40)
Mice	4	6	0	0
Rat	2	1	0	0

Fig.1. Perianal tape impressions showing eggs of oxyurids

Day 0 Day 21 Post treatment

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