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Eradication of Pinworms (*Syphacia spp.*) in a Laboratory Rodent Colony

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Abstract

Laboratory rodent colonies are commonly infested with pinworms namely *Syphacia obvelata* and *Syphacia muris,* often results in clinical signs such as diarrhea and rectal prolapse in heavy infestation. The pinworm infestation in rodents affects the experimental observations and hence it is important to eradicate them from the colony. Division of Laboratory Animals at Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST) maintains rats and mice colonies. Pinworm ova were determined by perianal impression with cellophane tape testing (CTT) under low power of a compound microscope. The mice and rats were divided into 2 groups with 10 males and 10 females in each group. Group I was treated with piperazine (10.8 mg/ml of drinking water) for a period of 28 days and group II was treated with piperazine (10.8 mg/ml) for a period of 14 days followed by ivermectin (0.01mg/ml) for 14 days. Cages and accessory equipments were disinfected with activated gluteraldehyde, 2.45% w/v. All supplies including bedding and accessories were autoclaved at 121^{o}C temperature, 15 lb pressure for 20 minutes. The present study revealed that the parasites were eradicated from those groups treated with combination drug (piperazine and ivermectin) therapy while the group treated with piperazine alone could not demonstrate a complete eradication. The two drug (piperazine and ivermectin) regime over a 4-week period, associated with appropriate sanitation programme was found to be effective in eradication of pin worms in laboratory rodents.

key words : *Syphacia muris*, rodents, piperazine, ivermectin

Introduction

Candidate materials used for the fabrication of biomedical devices such as heart valves, coronary stents, vascular grafts, orthopaedic devices, cardiotomy reservoirs, hydrocephalus shunts, blood pumps, oxygenators and blood bags undergo a battery of toxicological tests like acute systemic toxicity, hypersensitivity, hemocompatability, pyrogen testing and osteocompatability in smaller laboratory animal models. Laboratory rodents are an integral part of biomedical toxicological studies. Conventional laboratory rodent colonies are commonly infested with pinworms namely *Syphacia obvelata* and *Syphacia muris*. Most of the infestations are asymptomatic but complications like decreased weight, behavioral changes and altered immune response were often observed (Owen 1992; Baker 1998; Huerkamp *et al.* 2000). Clinical signs associated with heavy infestation are diarrhea, rectal prolapse, intussusceptions, and fecal impaction (Harkness and Wagner, 1989; Lubcke, 1992; Percy and Barthold 2001). The pinworms in rodents affect

the experimental observations and hence it is important to eradicate them from the colony.

These parasites are extremely difficult to eradicate, especially from laboratory rodent colonies. Caesarian rederivation or embryo transfer can be used to obtain parasite-free offspring in order to provide breeders for a new colony. A number of anthelminthics, including piperazine, ivermectin, trichlorfon, fenbendazole, dichlorvos, thiabendazole and mebendazole, have been used (Hoag 1961; Simmons *et a1*. 1965; Wagner 1970; MacArthur and Wood 1978; Baskerville *et a1*. 1988; Flynn *et a1*. 1989; Coghlan *et a1*. 1993 ; LeBlanc *et a1*. 1993). The number and frequency of these reports indicate a continuous search for a better therapy. In fact, only a few treatment regimens are reported to be successful for eradicating pinworms from large rodent breeding colonies.

This report describes an effective treatment protocol using piperazine and ivermectin combination for pinworm infestation in a medium sized rodent colony.

Materials and Methods

The present study was conducted in a medium sized laboratory rodent colony, bred and maintained at the Division of Laboratory Animals (DLAS), Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Science and Technology, Thiruvananthapuram, Kerala, India. The animal house is CPCSEA (Committee for the Purpose of Control and Supervision of Experiments in Animals) registered and the experimental protocols were approved and reviewed by Institutional Animal Ethics Committee. The rat colony consisted of two strains viz., Sctb: WI and Sctb: SD. Mouse colony consisted of Sctb: Swiss and BALB/c.

The rodents were examined for pinworm ova by perianal impression with cellophane tape as described by Dix *et al.* (2004) under low power (X10) compound microscope before the treatment. The sample size taken was as per the recommendations of FELASA (Kraft *et al*. 1994; Nicklas *et al*. 2002). Ten representative individuals from each strain were sampled. The age group split up was 4 retired breeders (>6 months), 4 young adults (10-14 weeks) and 2 weanlings (6-8 weeks) (Table I). A 25 x 150 mm cellophane tape was pressed against the area of pelt at peri-anal region and then affixed to a microscopic slide. The entire slide was examined thoroughly and systematically under X10 magnification to detect ova of *Syphacia spp*. (Fig.1). Treated animals were tape tested monthly after each treatment regime for four months to determine the recurrence of infestation and effectiveness of treatment.

Table I : Results of Cellophane Tape Test for Syphacia eggs before treatment

Strain/ Stock	Treatment Group I		Treatment Group II	
	Males (n=10)	Females (n=10)	Males (n=10)	Females (n=10)
Sctb:WI	8	9	9	10
Sctb:SD	10	7	8	8
Sctb:Swiss	8	7	6	8
BALB/c	8	8	8	9

Treatment

A randomized block design was made for assessment of treatment effectiveness in each stock/strain of animals. Each strain/stock of animal was divided into two groups with equal number of males and females. A total of 10 males and 10 females were selected from each strain/stock (Table 2).

Table 2 : Allocation of animals for treatment

Strain	Number of animals		Treatment Group I	Treatment Group II
	Males	Females		
Sctb:WI	10	10	Piperazine (10.8 mg/ ml) 0-28 days	Piperazine (10.8 mg/ ml) 0-14 days Ivermectin (0.01mg/ ml) 14-28 days
Sctb:SD	10	10		
Sctb: Swiss	10	10		
BALB/c	10	10		

Treatment group I was administered with piperazine alone for 28 days at 10.8 mg piperazine (piperazine hexahydrate, TTK Pharma) per ml of drinking water. Treatment group II was administered with 10.8 mg piperazine per ml of drinking water from day zero to day seven through drinking water. On day seven, drinking bottles were washed and filled again with piperazine and given until day 14. From day 14, 0.01 mg/ml ivermectin solution (ivermectin tablets, Neomec, INTAS) in drinking water was administered. On day 21, water bottles were washed and filled again with ivermectin and given until day 28.

Medicated water in each bottle lasted for one week, allowing animals to get continuous treatment. Complete cage changing was performed on day 28 for both the groups. Cages and accessory equipments were disinfected with activated gluteraldehyde, 2.45% w/v (Cidex, Johnson and Johnson). All supplies including bedding, and accessories were completely autoclaved in both the treatment groups.

Results

In the present study, treatment with two drugs (piperazine and ivermectin) combination over a 4-week period, associated with hygiene measures was chosen and tested in our animal facility.

Table 3 : Results of Cellophane Tape Test for Syphacia eggs, 28 days after treatment

Strain/ Stock	Treatment Group I		Treatment Group II	
	Males (n=10)	Females (n=10)	Males (n=10)	Females (n=10)
Sctb:WI	1	0	0	0
Sctb:SD	0	1	0	0
Sctb:Swiss	1	0	0	0
BALB/c	0	0	0	0

Syphacia infestation in piperazine alone group (group I) was not completely eradicated, which was evidenced by negligibly small proportion of animals in the colony tested positive although the number of ova found in these animals was very low. It was observed that animals in group II with two drug regimen is advantageous over group I animals (Table 3).

The present study showed that the oral administration of the combination of piperazine and ivermectin, associated with appropriate hygienic procedures, resulted in an inexpensive, safe and highly effective treatment for mouse and rat pinworms.

Fig. 1 : Eggs of Syphacia muris

Discussion

Prior to the treatment, the colony showed 87% positive results for *Syphacia spp*. The eggs (Fig.1) were identified by the descriptions given by Flynn (1989). The life cycles of these parasites are direct. Gravid female worms migrate from the caecum or the colon to the perianal region of the host, deposit embryonated eggs on the skin, then dry up and die. The eggs become infective within 6-24 h. The eggs hatch in the alimentary tract of another rodent and the larvae continue their way to the caecum where they moult through four stages to sexually mature adults. After fertilization, the males die and gets eliminated through faeces (Owen, 1992).

Only very few successful treatments were reported for the eradication of pinworms from rodent colonies. The control of *Syphacia spp*. infestation is difficult as most anthelminthics are only partially effective against adult worms and have no impact on larvae or ova; and also the eggs embryonate rapidly and can survive outside the host (Zenner, 1998). Treatment failures reported were commonly associated with reinfestation from eggs in the environment (Hoag, 1961). Lipman *et al.* (1994) published a report on effective treatment of *S. obvelata* in mice, using a combination of ivermectin and piperazine. We followed the same protocol in infested colonies of mice and rats but with modifications in dosage of piperazine (10.8 mg/ml) in drinking water when compared to 2.1 mg/ml of piperazine sulfate in drinking water by Lipman *et al*. (1994). However, we could find that the availability of piperazine hexahydrate as commercial medicine is ubiquitous when compared to piperazine sulfate in India. The dosage of 2.1 mg/ml of piperazine sulfate in drinking water by Lipman *et al*. 1994 could not be able to prevent recurrence of pin worm when compared to a higher dosage of piparazine hexahydrate (10.8 mg/ml in drinking water). An effective regimen must be based on the combination of adequate hygiene measures to remove parasite eggs from the environment, and chemotherapy with effective drugs at appropriate treatment intervals (Zenner, 1998).

The oral administration of piperazine citrate at a rate of 200-400 mg/kg for one week, followed by an interruption for a week and another treatment during the third week, as described by Hoag (1961), was used extensively. This protocol reduces the number of oxyurids present in mice colonies, but generally does not eradicate the parasites. Different treatment regimes with piperazine alone have proved unsuccessful in the eradication of pinworms (Lipman *et a1*. 1994) since viable eggs were detected from 2 to 4 months at the end of the treatment. Oral doses of ivermectin were effective against adults, gravid females and immature worms in mice (Flynn *et al.* 1985, Ostlind *et a1*. 1989). However, 3 weeks of treatment with ivermectin in water was ineffective for the treatment of a mouse colony infested with *S. obvelata,* since viable eggs were detected soon after treatment (Lipman *et a1. 1994).* Alternating the two regimens led to good results against *S. obvelata* in mice (Lipman *et a1*. 1994), but was neither tested in mice against *S. muris* nor in rats. The present treatment regime resulted in eradication of *Syphacia spp*. in the rodent colony. Results obtained after four months revealed that there was no re -infestation with the present two drug treatment regimen.

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