



## Prevalence of syphaciosis in laboratory rodents with emphasis on species identification, treatment and Th2 cytokine response

S T BINO SUNDAR<sup>1</sup>, T J HARIKRISHNAN<sup>2</sup>, BHASKARAN RAVI LATHA<sup>3</sup>, G SARATH CHANDRA<sup>4</sup>, T M A SENTHIL KUMAR<sup>5</sup>, S RAMESH<sup>6</sup>, M R SRINIVASAN<sup>7</sup>, P AZHAHIANAMBI<sup>8</sup>, S GOMATHINAYAGAM<sup>9</sup>, M RAMAN<sup>10</sup>, K G TIRUMURUGAAN<sup>11</sup> and SERMA SARAVANA PANDIAN<sup>12</sup>

Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu 600 051 India

Received: 5 October 2017; Accepted: 8 March 2018

### ABSTRACT

Prevalence of syphaciosis (pinworm infection) in laboratory rats and mice, species identification, chemotherapy with ivermectin and its effect on pinworm infection, and Th2 cytokine response was studied. Using perianal tape test examination, the highest rate of pinworm infection was found in male Wistar rats (69.44%) followed by female Wistar rats (62.50%), male Balb C mice (60.60%), female Swiss Albino mice (51.42%), male Swiss Albino mice (33.33%) and female Balb C mice (32.69%). Wistar rats had maximum infection (65.97%) followed by Balb C mice (46.64%) and Swiss albino mice (42.37%). Overall, males had higher infection (54.45%) compared to females (48.87%). *Syphacia obvelata* in mice and *S. muris* in rats were the pinworms identified morphologically and confirmed with polymerase chain reaction and sequencing. No mixed infection was found in rats and mice. Ivermectin (2.5 mg/kg) as oral gavage as well as in drinking water continuously for five days was highly effective in eradication of pinworms in rats and mice. Down regulation of Th2 cytokines, viz. IL4, IL5 and IL13 was observed after ivermectin treatment in both male and female Wistar rats.

**Key words:** Ivermectin, Rodents, *Syphacia obvelata*, Syphaciosis, Th2 cytokine

Pinworms are common inhabitants of the caecum and colon of rats and mice in laboratory animal facilities as well as in field conditions. They belong to superfamily Oxyuroidea, family Oxyuridae and genus *Syphacia*. The two common species under genus *Syphacia* are *S. obvelata* affecting mice and *S. muris* affecting rats. *Aspiculuris tetraptera* also infects both rats and mice and is found in the colon. Co-infections with multiple species of pinworms has been reported in rats and mice (Parel *et al.* 2008). Pinworms

of rodents have a direct life cycle involving retrofection or reinfection. Light weight eggs also spread rapidly by aerosolization leading to high environmental contamination. Syphaciosis is very common in specific pathogen free colonies as well. Infection with pinworms has untoward effects on growth, behaviour, intestinal physiology and immunology of laboratory rodents and is a major hinderance to the use of rodents in research especially immunological studies (Lubcke *et al.* 1992). Heavy infections cause rectal prolapse, catarrhal enteritis, intestinal impaction, sticky stools, intussusceptions, liver granuloma and perianal pruritus. Affected animals have an elevated Th2 cytokine profiles which may mislead the results of immunologic studies (Michels *et al.* 2006). Higher operational cost is also involved in treatment and decontamination of the pinworm infected lab animal facilities as well as surveillance and eradication of infections. The present study was undertaken to study the prevalence of pinworm infections in laboratory rats and mice, differentiate pinworms of rats and mice by morphology and molecular methods, study the efficacy of ivermectin in the treatment of pinworm infections, and assess the Th2 cytokine profile pre- and post-treatment with ivermectin.

### MATERIALS AND METHODS

*Laboratory animals:* The study was conducted at the

Present address: <sup>1,8</sup>Assistant Professor (binosundar.s.t@tanuvas.ac.in, azhahianambi.p@tanuvas.ac.in), <sup>3</sup>Professor and Head (bhaskaranravilatha@tanuvas.ac.in), <sup>5,11</sup>Professor (senthilkumar.t.m.a@tanuvas.ac.in, tirumurugaan.k.g@tanuvas.ac.in), Department of Animal Biotechnology; <sup>7</sup>Assistant Professor (srinivasan.m.r@tanuvas.ac.in), Department of Veterinary Pharmacology and Toxicology; <sup>9</sup>Professor (gomathinayagam.s@tanuvas.ac.in), Department of Veterinary Parasitology; <sup>12</sup>Assistant Professor (sermasaravanapandian.a@tanuvas.ac.in), Department of Animal Husbandry Economics, Madras Veterinary College, Chennai. <sup>2</sup>Director of Research (harikrishnan.t.j@tanuvas.ac.in), <sup>4</sup>Professor and Head (sarathchandra.g@tanuvas.ac.in), Pharmacovigilance Laboratory for Animal Feed and Food Safety; <sup>6</sup>Professor and Head (ramesh.s@tanuvas.ac.in), Laboratory Animal Medicine Unit, Directorate of Centre for Animal Health Studies; <sup>10</sup>Programme Head (Vaccines) (raman.m@tanuvas.ac.in), Translational Research Platform for Veterinary Biologicals.

Lab Animal Medicine Unit, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, India. Weaned male and female Wistar rats, Swiss Albino mice and Balb C mice were used for the study. The study was carried out with the approval under Committee for the Purpose of Control and Supervision of Experiments on Animals of TANUVAS (Approval No 1614/DFBS/B/2014) recommended by IAEC.

*Screening of rats and mice for pinworm infection by perianal tape test:* Rats (76) and mice (165) were screened for pinworm infection by the perianal tape test (Dole *et al.* 2011, Hill *et al.* 2009). Animals were restrained. Cellophane tape (1 cm width) was applied to the perianal region and taken back several times until stickiness was lost. The tape was pasted to a clean grease free glass microscopic slide. A 3 × 1 cm area in the middle of tape was marked and examined under low power of microscope for pinworm eggs. Eggs were identified by the morphological characters and counted. Overall prevalence as well as sex wise and breed wise prevalence of pinworm infection was determined. The method was very easy to perform and enabled on the spot screening and segregation of infected rats and mice to separate cages.

*Estimation of haematological parameters in Wistar rats positive for pinworms:* Blood smears were prepared from pinworm positive Wistar rats and subjected to a complete blood count and differential count. The blood counts were made using haemocytometer.

*Differentiation of Syphacia sp. by morphological characteristics of eggs:* Eggs obtained in tape test were measured and observed under 10× of the microscope and morphological characters were studied.

*Differentiation of Syphacia sp. by morphological characteristics of worms:* Live pinworms were collected from caecum and colon of Swiss Albino mice (5) and Wistar rats (5) during necropsy were directly observed under 5× and 10× of the microscope and morphological characters were studied.

*Differentiation of Syphacia sp. by polymerase chain reaction:* Worms collected from rats and mice were washed twice thoroughly in PBS and stored at -80°C. Genomic DNA was isolated by phenol chloroform method from 10 worms (pooled sample) with an initial dip in liquid nitrogen for 15 min. PCR was performed using genus and species specific primers (Sigma) as per Parel *et al.* (2008) (Table 1). PCR was carried out in the thermocycler (Biorad) in a total volume of 20.0 µl comprising 4.8 µl nuclease free water, 2.0 µl DNA template, 1.0 µl (10 pmol) of forward

primer, 1.0 µl (10 pmol) of reverse primer, 10.0 µl of PCR master mix (containing 1.5 mM MgCl<sub>2</sub>), and 1.2 µl of 3 mM MgCl<sub>2</sub>. Reaction conditions for PCR consisted of an initial denaturation at 95°C for 3 min followed by denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec and a final extension at 72°C for 5 min. The PCR product was run in a 2% agarose gel and viewed under Gel Documentation system (Biorad). For further confirmation of species, PCR-RFLP was done using RsaI (*Rhodopseudomonas sphaeroides*) enzyme in a reaction volume of 15 µl comprising 3.5 µl of nuclease free water, 1.5 µl of RsaI buffer (10×), 8.0 µl of PCR product and 2.0 µl of RsaI. The mixture was incubated for 4 h at 37°C and the product was run in a 2% agarose gel and viewed.

*Efficacy of ivermectin for treatment of pinworm infections:* Rats and mice found positive for pinworm eggs in perianal tape test were segregated into five groups (Group I to V) each comprising five animals, marked with picric acid and kept in separate cages for administration of ivermectin. Picric acid marking was made in head, body, tail, right forelimb and one animal was left unmarked. Cages were also marked separately. Ivermectin (Silvotin, 0.1%, Sihil Pharma @ 2.5 mg/kg) in a dose volume of 10 ml/kg b.wt. was administered to Wistar rats as oral gavage (Group I) and in drinking water (Group II). Ivermectin was administered in drinking water for Swiss Albino mice (Group III) and Balb C mice (Group IV). Dosing for all groups was done for five days continuously. Ivermectin preparation was freshly made each day and administered immediately to the animals. Control group (Group V) was administered with water. Tape tests were performed one day pre-dosing and on day 1, 14 and 28 post dosing for pinworm eggs. Body weight was measured before dosing and on day 1, 14 and 28 after dosing. Cage wise daily feed intake and water consumption was monitored in order to find out whether medication with ivermectin had any adverse effect on feed and water intake.

*Assessment of Th2 cytokine profile pre- and post-treatment with ivermectin administered as oral gavage in Wistar rats:* Blood samples (300 µl) were collected from male and female Wistar rats (n=10 comprising five males and five females) by retro orbital plexus puncture pre- and post-treatment (one day before start of treatment and next day after completion of the 5 day dosing) with ivermectin as oral gavage. RNA was isolated and cDNA was prepared and stored at -80°C and RT-PCR was done using Roche Light cycler®. Expression levels of Th2 cytokines (IL4, IL5 and IL13) were estimated with β actin as control pre-

Table 1. Primers used in PCR for differentiation of pinworm species

Species of pinworm	Forward primer	Reverse primer
Genus specific	NC5- GTA GGT GAA CCT GCG GAA GGA TCA TT	NC2 -TTA GTT TCT TTT CCT CCG CT
<i>S. obvelata</i>	SoF-ACA AAT TAA AGT TGT CGA CTG ACT G	NC2 -TTA GTT TCT TTT CCT CCG CT
<i>S. muris</i>	SmF-CCT ATG ACG ATG GCA TGT TC	NC2 -TTA GTT TCT TTT CCT CCG CT
<i>A. tetraptera</i>	AsF-ATA CTC TTT AAC GCA TAC AC	AsR-TGC GGC CTA CAG TAA AAA GC

Table 2. Primers used in RT-PCR for estimating Th2 cytokine levels

Cytokine	Forward primer	Reverse primer
IL4	CGT CAC TGA CTG TAG AGA GC	GGG CTG TCG TTA CAT CCG
IL5	TGC TTC TGT GCT TGA ACG TTC TAA C	TTC TCT TTT TGT CCG TCA ATG TAT TTC
IL13	CTT GCC TTG GTG GTC TTG	TCT TCT GGT CTT GTG TGA TG
$\beta$ actin	ACT ATC GGC AAT GAG CGG TTC C	CTG TGT TGG CAT AGA GGT CTT TAC G

and post-dosing with ivermectin using specific primers (Table 2) as per El-Nagger *et al.* (1998). RT-PCR was performed in a total volume of 10  $\mu$ l containing 1.0  $\mu$ l of DNA template, 0.5  $\mu$ l of forward primer, 0.5  $\mu$ l of reverse primer, 5.0  $\mu$ l of PCR SyBr Green Mastermix and 3.0  $\mu$ l of nuclease free water. Reaction conditions for PCR consisted of one initial cycle of denaturation at 95°C for 30 sec followed by 40 cycles of denaturation at 95°C for 5 sec and optimized annealing temperature (annealing/extension) at 60°C for 60 sec for 40 cycles followed by 95°C for 20 sec, 65°C for 60 sec, 95°C for 1 sec: Melt curve (2–5 sec/step) (1 cycle). CQ (CT) values were obtained and expression of interleukin levels was calculated based on the fold change ( $2^{\Delta\Delta CT}$ ).  $\Delta\Delta CT = \Delta CT1 - \Delta CT2$  ( $\Delta CT1 = CT (IL AT) - CT (\beta Actin AT)$  and  $\Delta CT2 = CT (IL BT) - CT (\beta Actin BT)$ ). BT indicates CQ (CT) values before treatment and AT indicates CQ (CT) values after treatment.

**Statistical analysis:** Data analysis was carried out by Chi-square test using SPSS software (SPSS 20.0, Inc., Chicago, Illinois, USA).

## RESULTS AND DISCUSSION

**Prevalence of pinworm infections in laboratory rats and mice:** The highest rate of pinworm infection was found in male Wistar rats (69.44%) followed by female Wistar rats (62.50%), male Balb C mice (60.60%), female Swiss Albino mice (51.42%), male Swiss Albino mice (33.33%) and female Balb C mice (32.69%) (Table 3). Number of eggs in perianal tape test ranged from a single egg to several hundred in clumps. Sex-wise prevalence revealed a higher infection in males (54.45%) compared to females (48.87%) although the variation was not significant statistically ( $\chi^2$ : 0.70<sup>NS</sup>). Amongst the breeds, incidence was highest in Wistar rats (65.97%) followed by Balb C mice (46.64%) and Swiss Albino mice (42.37%) which was highly significant statistically ( $\chi^2$ : 11.45<sup>\*\*HS</sup>).

Table 3. Prevalence of pinworm infections in laboratory rats and mice

Species	Sex	No. screened	No. positive for pinworm eggs	% of infection
Wistar rats	Male	36	25	69.44
Wistar rats	Female	40	25	62.50
Swiss Albino mice	Male	45	15	33.33
Swiss Albino mice	Female	35	18	51.42
Balb C mice	Male	33	20	60.60
Balb C mice	Female	52	17	32.69

Experimental murine models are highly informative in understanding the factors contributing to resistance or susceptibility of diseases in both humans and domesticated animals (Grencis 2011). However, the importance of sterility in housing and transporting laboratory animals is sometimes underestimated, potentially increasing the vulnerability of laboratory rats and mice to adventitious pathogens (Reuter and Dysko 2003).

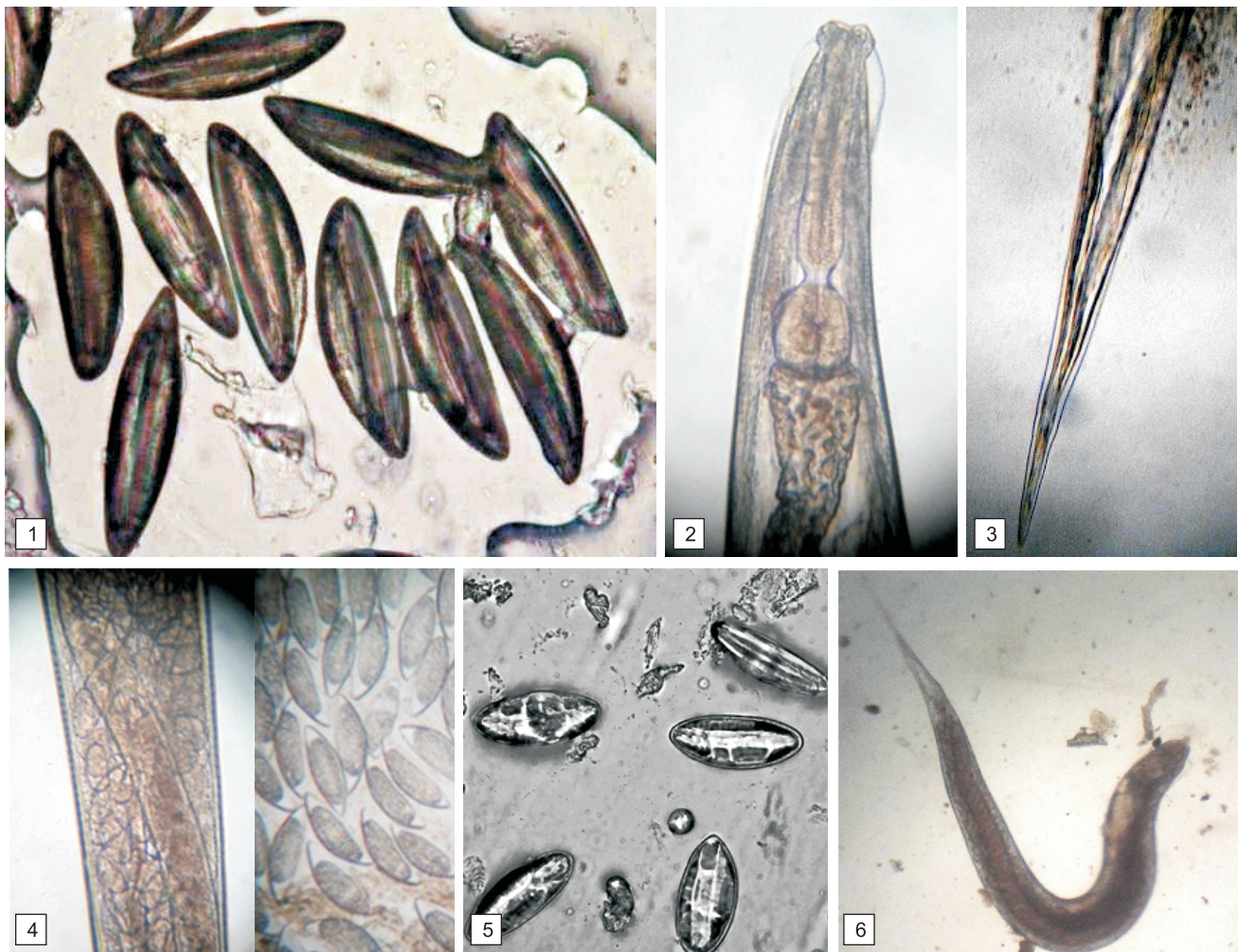
Malsawmtluangi and Tandon (2009) reported the occurrence of *Syphacia* sp. in 17.32% of rats and 9.09% of mice in Mizoram, while Milazzo *et al.* (2010) observed comparatively lesser infection in rats compared to mice where the prevalence of *S.obvelata* in mice was 23.29% and that of *S. muris* in rats was 19.51%. Similar observations were also made by Pakdel *et al.* (2013) who observed that infection with *S.obvelata* and *S. muris* in mice (*Mus musculus*) was 14.54% and 4.54%, respectively whereas infection with *S. obvelata* was 4.34% in *Rattus norvegicus* and 20% in *Rattus rattus*. Investigation by Chen *et al.* (2011) on pinworm infections in laboratory Kunming mice in two centres in Southern China indicated that 97% and 45.5% of mice, respectively were infected with pinworms.

Sexwise variations were also observed in the present study where male animals had higher infection rate compared to females. Dole *et al.* (2011) reported that male mice are more susceptible to pinworm infection than females which corroborates with the present findings. No mixed infections were observed in both rats and mice.

**Haematological changes in pinworm infected Wistar rats:** Complete blood count results indicated that the count of all cells was within the reference range in all infected animals but hypochromasia was observed in two male and two female Wistar rats and leucopenia was observed in one male Wistar rat.

**Morphological differentiation of pinworm species in rats and mice:** Two species of pinworms were identified in the current study. Rats were infected with *S. muris* and mice were infected with *S. obvelata*. No mixed infection was observed in rats and mice. Eggs of *S. obvelata* were thin shelled, banana shaped, elongated with a left-right asymmetry (Fig. 1).

The average size of eggs was 130.79  $\times$  44.17  $\mu$ m in Swiss albino mice and 123.46  $\times$  39.99  $\mu$ m in Balb C mice. *S. obvelata* adult worms were recovered from Swiss Albino mice during post mortem examination. The worms were found predominantly in the caecum. Only female worms were recovered and they measured 3–5 mm in length. The head end had a small cervical alae and a round oesophageal bulb (Fig. 2). The tail was long and pointed (Fig. 3) and the



Figs 1–6. 1. Eggs of *Syphacia obvelata* (40×); 2. *Syphacia obvelata* cranial end (5×); 3. *Syphacia obvelata* caudal end (5×); 4. *Syphacia obvelata* coiled uterus and eggs (5×); 5. Eggs of *Syphacia muris* (40×); 6. *Syphacia muris* cranial end with larger cervical alae (5×).

highly coiled uterus was filled with the characteristic eggs (Fig. 4). Eggs of *S. muris* were thin shelled, ellipsoidal and flattened on one side. The average size of eggs of *S. muris* was  $79.60 \times 33.38 \mu\text{m}$  in Wistar rats (Fig. 5). The worms were found predominantly in the colon of Wistar rats. Adult females measured 3–4 mm in length and had a comparatively larger cervical alae (Fig. 6), round oesophageal bulb and egg filled uterus. Other morphological features were similar to that of *S. obvelata*.

Adult pinworms of rats and mice are morphologically identical, however morphology and morphometry of eggs of these two species differ. Eggs of *S. obvelata* are thin shelled, banana shaped with left-right asymmetry ranging from  $118\text{--}151 \times 33\text{--}55 \mu\text{m}$ , but eggs of *S. muris* are smaller than that of *S. obvelata*, thin shelled, ellipsoidal and flattened to one side with dimensions of  $72\text{--}82 \times 25\text{--}36 \mu\text{m}$  (Dole *et al.* 2011, Pakdel *et al.* 2013 and Hitt *et al.* 2009). In the present study even though the morphological characteristics of adult worms and eggs matched with the above reports, cervical alae of female *S. muris* worms obtained from Wistar rats was slightly broader than that of the cervical alae of *S. obvelata* obtained from Swiss Albino mice.

*PCR based differentiation of pinworm species in rats and mice:* Primary PCR revealed band at approximately 800 bp indicating that the worm belongs to Genus *Syphacia* and species specific PCR revealed amplification at ~350 bp confirming the identity of *S. obvelata* (Fig. 7). Further, PCR-RFLP using *RsaI* enzyme revealed two clear bands one at 450 bp and other at 339 bp specific to *S. obvelata* (Fig. 8).

*Sequencing of Syphacia obvelata:* Sequencing results confirmed *S. obvelata* and it was 98% identical to the European strain. *S. obvelata* also had a close identity with the human pinworm or seat worm *Enterobius vermicularis* (GenBank Accession no: KT853017) (Fig. 9).

PCR based technique for speciation of rodent pinworms was designed by Hitt *et al.* (2009), Dole *et al.* (2011) and Parel *et al.* (2008). In the present study, the species specific primers of *S. obvelata* successfully amplified ~350 bp segment and RFLP with *RsaI* enzyme produced two fragments (~450 bp and ~339 bp) and this was in accordance with the findings of Parel *et al.* (2008). Further, sequencing of *S. obvelata* indicated its close identity with the European strain and *Enterobius vermicularis*.

*Efficacy of ivermectin in treatment of pinworm infections*

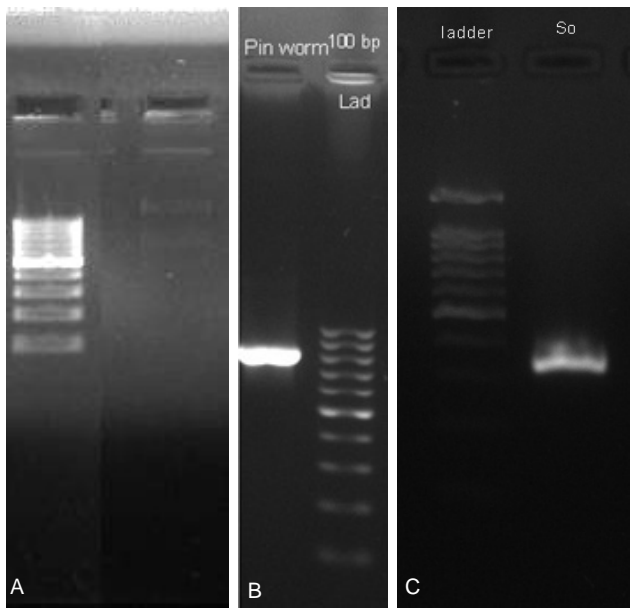


Fig. 7. PCR amplification of DNA from *Syphacia obvelata*. A, Genomic DNA; B, Primary PCR; C, Nested PCR band at ~350 bp.

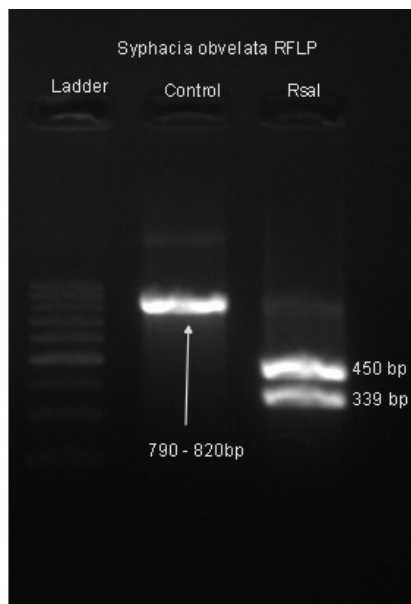


Fig. 8. PCR-RFLP of *Syphacia obvelata* DNA using *RsaI* enzyme.

Table 4. Interleukin expression fold change ( $2^{\Delta\Delta CT}$ ) in Wistar rats after treatment with ivermectin @2.5 mg/kg b.wt. as oral gavage continuously for five days

Animal no	IL4	IL5	IL13
4 (Male)	0.44	0.05	0.15
5 (Male)	0.23	4.35	0.54
3 (Male)	0.46	0.00	1.00
8 (Female)	0.23	0.06	1.42
9 (Female)	0.46	0.00	0.11
10 (Female)	1.05	0.15	0.19

*in rats and mice:* In male Wistar rats, ivermectin administered as oral gavage caused total elimination of worms as indicated by no egg in tape test results from day 1 till day 28 post dosing. Only one animal had eggs in tape

test on day 1 post dosing. Female Wistar rats were also negative for eggs in tape test on day 1, 14 and 28 post dosing. Similar effects were also observed in Wistar rats, Swiss Albino mice and Balb C mice administered ivermectin in drinking water. Oral ivermectin therapy has been recommended to be most ideal for treatment and eradication of pinworm infections in rodents (Lorcheim 2013, Klement *et al.* 1996 and Lytvynets *et al.* 2010). In the present study, administration of 2.5 mg/kg of ivermectin as oral gavage as well as in drinking for 5 days continuously was very successful in reducing the worm load as indicated by absence of eggs in tape test till day 28 post dosing confirming the above reports. Ivermectin was also well tolerated. According to Trailovic and Nedeljkovic (2011), ivermectin did not produce any neurotoxic effects at 2.5, 5.0 and 7.5 mg/kg when administered intravenously. However, Lankas *et al.* (1989) observed toxic effects in neonatal rats administered with 3.6 mg/kg of ivermectin orally. In this study all animals used were adults and ivermectin did not cause any harmful effects on them.

*Th2 cytokine profiling before and after treatment with ivermectin in Wistar rats:* Expression levels of Th2 cytokines, viz. IL4, IL5 and IL13 were reduced in pinworm infected Wistar rats treated with ivermectin as oral gavage for five days compared to the levels before treatment. However, there was no significant difference in the expression levels between the three interleukins after dosing (Table 4). One of the major hindrance in using pinworm infected rats and mice for immunological research lies with the altered levels of Th2 cytokine profiles. Infection with *S. obvelata* induces protective Th2 immune responses and affected animals normally will have elevated levels of Th2 cytokines namely IL4, IL5 and IL13. Th2 cell responses are highly essential to mediate resistance and worm expulsion (Michels *et al.* 2006) and it was observed that pinworm infections influence the outcome of experimental results. In the present study, estimation of Th2 cytokine profiles pre- and post-dosing with ivermectin as oral gavage for five days in *S. muris* infected male and female Wistar rats revealed that all the infected animals had an elevated levels of IL4, IL5 and IL13 indicating an upregulation which was downregulated after ivermectin therapy. Only limited studies are available regarding the effect of anthelmintic medications like ivermectin on Th2 cytokine levels pre- and post-dosing in pinworm infected rats but certain studies on effect of Fenbendazole on immune responses like levels of T and B cell markers in spleen and T cell markers in thymus of mice indicate no significant changes before and after treatment (Cray *et al.* 2008).

The present study was undertaken to find out the prevalence of syphaciosis (pinworm infection) in laboratory rodents. Among rats and mice, the highest prevalence of pinworm infection was found in male Wistar rats (69.44%) whereas female Balb C female mice had the lowest prevalence (33.69%). Infection was higher in males than females. Wistar rats had maximum infection (65.97%) followed by Balb C mice (46.64%) and Swiss albino mice

```

1 aatacattca aaaatgaatt aaagaagcta atttcttaca tgctataaac gtaagaaata
61 tagggcaaag atactcgcgc ttgtgcagat gcatgaggcg tttgaaagat ctgcgtcgat
121 gcaagaagtg gacgaggtat atggttggtg tctagcctga tttcttghtaa attagatgt
181 gccgcaaca aagcggaaga ctcaattagc gcagtcaagc gcgagccaa caattgaaa
241 tattatgaaa atattaactg tatggctacg tttgataatc gtagcatata agaaataaac
301 tcctagcggg ggatcactag gctcgaagat cgatgaagaa cgcagttagc tgcgataatt
361 agtgtgaatt gcggacacat tgaacacaaa aaattcgaac gcacattgcy ccacgggctc
421 ctctcccgtt ggtacgtctg gttgagggtt gaaatctaca aattaaagtt gtcgactgac
481 tgctaataata tacatgcact cagtttagta tgtttagggt gcttatagct accacaaaac
541 acgtaatgtg atgtggtgac ataacgacaa cgattttgca tggcaagtat agtcgtgctg
601 cgttgtgcaa gtggccgaac tctgttaggc atctgcacat gaaaccaagg tagatgctta
661 gcgggatcgg tatgacgtat ctgtgatact atagcaaaa catgtctgtc aaccacgtta
721 tgataacctc aactcagtcg tgatacccgc tga

```

Fig. 9. *Syphacia obvelata* isolate MVC internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

(42.37%). Two species of pinworms, viz. *S. obvelata* in mice and *S. muris* in rats were identified based on morphological characteristics, polymerase chain reaction and sequencing. Ivermectin (2.5 mg/kg) administered orally as gavage as well as in drinking water continuously for five days was highly effective in eradication of pinworms in rats and mice. Downregulation of the Th2 cytokines namely IL4, IL5 and IL13 was observed after treatment with ivermectin in both male and female Wistar rats.

#### REFERENCES

- Chen X M, Li X, Lin R Q, Deng J U, Fan W Y, Yuan Z G, Liao M and Zhu X Q. 2011. Pinworm infection in laboratory mice in southern China. *Laboratory Animals* **45**: 58–60.
- Cray C, Villar D, Zaias J and Altman N H. 2008. Effects of fenbendazole on routine immune response parameters of BALB/c mice. *Journal of the American Association for Laboratory Animal Science* **47**: 32–36.
- Dole S V, Zaias J, Kyricopoulos-Cleasby D M, Banu L A, Waterman L L, Sanders K and Henderson K S. 2011. Comparison of traditional and PCR methods during screening for confirmation of *Aspicularis tetraptera* in a mouse facility. *Journal of the American Association for Laboratory Animal Science* **50**: 904–09.
- El-Naggar M M, Ukai K, Takeuchi K and Sakakura Y. 1998. Expression of interferon-gamma, interleukin-4 and interleukin-5 mRNA in the nasal mucosal membrane of rats with allergic rhinitis. *Scandinavian Journal of Immunology* **47**: 554–60.
- Grencis R K. 2001. Cytokine regulation of resistance and susceptibility to intestinal nematode infection—from host to parasite. *Veterinary Parasitology* **100**: 45–50.
- Hill W A, Randolph M M and Mandrell T D. 2009. Sensitivity of perianal tape impressions to diagnose pinworm (*Syphacia* spp.) infections in rats (*Rattus norvegicus*) and mice (*Mus musculus*). *Journal of the American Association for Laboratory Animal Science* **48**: 370–80.
- Hitt K A, Riley L K and Livingston R S. 2009. Detection of three pinworm species in laboratory mice and rats through an antemortem fecal PCR assay. Project Report of Research Animal Diagnostic Laboratory, University of Missouri funded by Glaxosmithkline and the American Society of Laboratory Animal Practitioners.
- Klement P, Augustine J M and Delaney K H. 1996. An oral ivermectin regimen that eradicates pinworms (*Syphacia* spp.) in laboratory rats and mice. *Laboratory Animal Science* **46**: 286–90.
- Lankas G R, Minsker D H and Robertson R T. 1989. Effects of ivermectin on reproduction and neonatal toxicity in rats. *Food and Chemical Toxicology* **27**: 523–29.
- Lorcheim K. 2013. LAS Overview. The clinical and environmental treatment of pinworms and their eggs. *Laboratory Animal Science Professional*. pp 58–61.
- Lubcke R, Hutchenson F A R and Barbezat G O. 1992. Impaired intestinal electrolyte transport in rats infested with the common parasite *Syphacia muris*. *Digestive Diseases and Sciences* **37**: 60–64.
- Lytvynets A, Langrova I, Lachout J, Vadlejch J, Fucikova A and Jankovska I. 2010. Drinking water ivermectin treatment for eradication of pinworm infections. *Helminthologia* **47**: 233–37.
- Malsawmtluangi C and Tandon V. 2009. Helminth parasite spectrum in rodent hosts from bamboo growing areas of Mizoram, North-east India. *Journal of Parasitic Diseases* **33**: 28–35.
- Michels C, Goyal P, Nieuwenhuizen N and Brombacher F. 2006. Infection with *Syphacia obvelata* (Pinworm) induces protective Th2 immune responses and influences ovalbumin-induced allergic reactions. *Infection and Immunity* **74**: 5926–32.
- Milazzo C, Cagnin M, Dibella C, Geraci F and Ribas A. 2010. Helminth fauna of commensal rodents, *Mus musculus* (Linnaeus, 1758) and *Rattus rattus* (Linnaeus, 1758) (Rodentia, Muridae) in Sicily (Italy). *Revista Ibero-Latinoamericana de Parasitología* **69**: 194–98.
- Pakdel N, Naem S, Rezaei F and Chalehchaleh A. 2013. A survey on helminthic infection in mice (*Mus musculus*) and rats (*Rattus norvegicus* and *Rattus rattus*) in Kermanshah. *Iran Veterinary Research Forum* **4**: 105–09.
- Parel J D, Galula J U and Ooi H K. 2008. Characterization of rDNA sequences from *Syphacia obvelata*, *Syphacia muris* and *Aspicularis tetraptera* and development of a PCR-based method for identification. *Veterinary Parasitology* **153**: 379–83.
- Reuter J D and Dysko R C. 2003. Quality assurance/surveillance monitoring programs for rodent colonies, pp 1–14. *Laboratory Animal Medicine and Management*. (Eds) Reute J D and Suckow M A. International Veterinary Information Service, Ithaca, New York.
- S M Trailovic and Nedeljkovic J T. 2011. Central and peripheral neurotoxic effects of ivermectin in rats. *Journal of Veterinary Medical Science* **73**: 591–99.