

TREATMENT OF ACARIASIS WITH IVERMECTIN AND EVALUATION OF DIFFERENT SAMPLING TECHNIQUES IN MICE

Alenka Dovč¹, Renata Lindtner-Knific¹, Ines Markelc², Aleksandra Vergles Rataj³, Maja Gorišek Bajc³, Petra Zrimšek⁴, Marina Pavlak⁵, Petra Isaković¹, Ksenija Vlahović⁶

¹Institute for Health Care of Poultry, ³Institute for Microbiology and Parasitology, ⁴Clinic for Reproduction and Horses, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, ²Jele Kitt. d.o.o., Pot v Jele 5, 1353 Borovnica, Slovenia, ⁵Department of Veterinary Economics and Epidemiology, ⁶Department of Biology, Veterinary Faculty, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

*Corresponding author, E-mail: alenka.dovc@vf.uni-lj.si

Summary: Breeding mice positive for *Myocoptes musculus* and *Myobia musculi* were treated with ivermectin in doses of 200 µg/kg of body weight (6 µl of active substance) as a spot application on the back of the neck. Application was repeated three times in seven-day intervals. Different development forms (adults, nymphs, larvae, and eggs) were observed in groups of adult and young mice before the treatment as well as during and after the treatment.

Before treatment, the fur pluck technique and sticky paper technique (sampling from two different places: from back and abdomen) were evaluated. The fur pluck technique was used as the gold standard. The sticky paper technique has 91.5 to 93.2% sensitivity for *Myocoptes musculus* and 10.8 to 13.5% for *Myobia musculi*. We observed that *Myobia musculi* place eggs next to the skin, on the bases of two or more hairs together; consequently, the sampling is not always satisfactory.

The effectiveness of therapy with ivermectin for *Myocoptes musculus* and *Myobia musculi* was shown after the second treatment. Before the treatment, as well as during and after the treatment, a significantly higher percentage of positive mice was observed among old than in young ones. After the third treatment, adults and developmental stages (eggs, larvae, and nymphs) were still found only in *Myocoptes musculus*. Almost all eggs of *Myocoptes musculus* and *Myobia musculi* were drained and damaged after the third treatment.

Key words: veterinary science; mice; ectoparasites; *Myocoptes musculus*; *Myobia musculi*; treatment

Introduction

Fur mites are a persistent problem in mouse colonies, especially in crowded laboratory colonies or in animals in poor condition (1). Murine acariasis is frequently caused by *Myocoptes musculus*, *Myobia musculi* and *Radfordia affinis* (2, 3). Fur mites are primarily transmitted through direct mouse-to-mouse contact (1). The most frequently published antiparasitic drugs for the treatment of mites in rodents are ivermectin

(4, 5, 6, 7, 8), selamectin (9, 10) and moxidectin (10). However, since the 1990s, mite infections have been controlled with different acaricides, including neguvon (4, 8), dichlorvos, permethrin, pyrethrins and trichlorfon (9).

Most protocols describing effective fur mite eradication require individual treatment of animals (9) or the preparation of medicated water bottles (4, 11). Arbona et al. (6, 7) evaluated the administration of an oral acaricide in feed by using ivermectin-compounded feed in a large colony with over 120,000 mice. Such treatment was very useful for large colonies but for small colonies individual treatment is more frequently

used. Metcalf Pate et al. (3) reported different successful eradication of fur mites with topical parasiticides, oral drugs, ivermectin-impregnated feed, injectable ivermectin, environmental agents and cross-fostering paired with ivermectin treatment. Gonenc et al. (9) emphasized that the choice of acaricide, method of application and treatment interval are essential in the control of these ectoparasites.

The degree of pathogenicity to mice is variable among strains and even in single colonies (9). Fur mites can cause clinical signs in infested mice, which include localized pruritus, alopecia, ulcerative dermatitis, lymphadenopathy, weight loss (2), auto-traumatization and secondary amyloidosis (12).

The identification and eradication of murine fur mite infestations are ongoing challenges faced by many research institutions (2) and other mouse-breeding facilities, including pet shops. Roble et al. (13) investigated the prevalence of ectoparasites, endoparasites and infectious agents carried by apparently healthy mice obtained from pet stores.

In our research, a mouse breed intended for pet stores or as a reptile food was treated for mites. The most commonly used concentration of ivermectin (200 µg/kg body weight (BW)) (5, 6) was used. Three different techniques were used, and the results had been compared between fur pluck technique, sticky paper technique from the back and sticky paper technique from the abdomen. The fur pluck technique was the most sensitive technique; therefore, it was used to monitor the effect of treatment with ivermectin.

Material and methods

Animals

A colony of white mice consisted of six large terrariums with a group of 500 mice in each (reproductive units). The ratio of bucks and does in boxes is about 1:20 in favour of females. A few days before giving birth, mice are moved into small terrariums (nursery units). Ten to twenty females reside together with their baby mice until their removal at the age of 20 days (units intended for sale).

A group of 59 mice in nursery units were treated with Ivermectin 10, solution for injection (1 % ivermectin – 10 mg/ml – 1 % w/v). From twelve nursery units three, with 10, 14 and 15 does

were chosen randomly. Baby mice involved in the investigation originated only from one unit with 10 mice. Ivermectin was diluted 1:50 with a mixture of equal amounts of propylene glycol and water to obtain a 0.02 % concentration of the active ingredient (200 µg/kg BW). Treatment of 39 does (30 g BW), and 20 young mice, three weeks old (10 g BW) was performed. Does were given 30 µl diluted preparation and young mice 10 µl. The Ivermectin solution was placed as a spot application on the back of the neck. Application was repeated three times in seven-day intervals. Pipette and tips (Biohit pipette 5–25 µl) were used for the precise dosing of the drug. These animals were monitored individually throughout the experiment: before, during and after the treatment.

Sampling procedure

Before treatment, the samples of fur were taken in different ways, from different places, and divided into three groups. Fur was collected via the impression of 3 cm long duct tape on the back (sticky paper technique on the back), the impression of 3 cm long duct tape on the abdomen (sticky paper technique on the abdomen) and by pulling hair from head, back and abdomen and combined (fur pluck technique). The samples were always taken in the same order; sticking on the back, sticking on the abdomen and pulling. Control for the presence of ectoparasites was performed one day before starting the treatment and monitored during the therapy before every administration of ivermectin. Based on the results of evaluating the sampling techniques, during and after treatment, the samples of hair were taken only with the fur pluck technique.

Evaluation of different sampling techniques

The fur pluck technique was used as the gold standard in defining the sensitivity of both sticky paper techniques for the detection of *Myocoptes musculinus*. The same procedure was used to evaluate the diagnostic potential for detecting *Myobia musculi* by all three techniques.

Data were analysed using Excel 2002 software. Differences among groups were analysed using Chi-squared / Fisher exact test with IBM SPSS Statistics program, version 20. Values of $P < 0.05$ were considered to be significant.

Parasitological examination

Ectoparasites were examined under the stereomicroscope at 10× and 16× magnification. Samples obtained by pulling hair (fur pluck technique) or with impression of 3 cm long duct tape (sticky paper technique) were examined. Details of mites or their eggs were observed under a microscope at 40× and 100× magnification. Samples were previously soaked with 10 % KOH. Monitoring the presence of ectoparasites (adults, nymphs, larvae, and eggs) before, during and after the treatment was performed.

Results

Evaluation and the usefulness of different sampling techniques

Before treatment, all 59 (100 %) examined mice were confirmed to be positive for *Myocoptes musculus* via the fur pluck technique. When we used sticky paper technique on the back and abdomen, 55 (93.2 %) and 54 (91.5 %) mice, respectively, were confirmed to be positive for *Myocoptes musculus* (Table 1). The sensitivity of the sticky paper technique on the back and abdomen for *Myocoptes musculus* was 91.5 and 93.2 %, respectively.

Before treatment, 37 (62.7 %) out of 59 examined mice were confirmed to be positive for *Myobia muscili* via the fur pluck technique. *Myobia muscili* was found on the backs of five mice (8.5 %) and on the abdomens of four mice (6.8 %) by using the sticky paper technique (Table 1). The results reveal the low sensitivity of the sticky paper technique (10.8 and 13.5 %, respectively) for *Myobia muscili*.

Eggs of *Myocoptes musculus* were confirmed in all animals via the fur pluck technique before treatment, while it was determined in 89.8 % of the mice using sticky paper techniques. A total of 28.8 % of adults, nymphs, larvae and eggs were detected with the fur pluck technique and only 3.4 % to 8.5 % with sticky paper techniques. Eggs of *Myobia muscili* were confirmed in 61.1 % of animals via the fur pluck technique before treatment. Smaller percentages (from 6.8 % to 8.5 %) were determined via sticky paper techniques. Adults and their development forms were confirmed only via the fur pluck technique in 13.6 % (Table 2).

The effectiveness of therapy with ivermectin

Treatment of 59 mice was performed individually. Before, during and after the treatment, adults (N=39) and young mice (N=20) were separately monitored. Ivermectin therapy was monitored via parasitological examination, using the fur pluck technique. Table 3 shows the number of infested mice considering the age of mice.

The effectiveness of therapy with ivermectin for *Myocoptes musculus* is statistically verifiable. A significant difference ($P < 0.05$) in the proportion of infested mice after the second (75.4 %) and after the third (78.9 %) week of therapy was found in comparison with the number of infested mice with *Myocoptes musculus* before treatment (100 %). The effectiveness of therapy against *Myobia muscili* was better than against *Myocoptes musculus*; after the second and third weeks, only 24.3 and 35.1 % of mice, respectively, were still infested with *Myobia muscili*. The effectiveness of therapy with ivermectin is not evident after the first treatment for *Myocoptes musculus* ($P = 0.496$), nor is it for *Myobia muscili* ($P = 0.396$). A significant decrease in the number of infested mice with *Myocoptes musculus* is observed between the first and second (from 57 to 43) and between the first and third treatments (from 57 to 45) ($P < 0.001$). The proportion of mice infested with *Myocoptes musculus* and *Myobia muscili* did not differ from the second to the third week of therapy ($P = 0.414$ and $P = 0.153$, respectively).

All adult and young mice were infested with *Myocoptes musculus* before the treatment, while a significantly lower proportion ($P < 0.001$) of young mice (30.0 %) than adult mice (79.5 %) were infested with *Myobia muscili*. No difference in the proportion of infested mice with *Myocoptes musculus* between adult and young mice was observed after the first treatment, but after the second and third treatments the proportion of infested mice was lower in young (31.6 and 36.8 %, respectively) than in adult mice (97.3 and 100.0 %, respectively). We found that even after the third treatment, all examined adult animals were still infested (mainly drained and damaged eggs were present, which cannot be seen from Table 3). Only eggs of *Myocoptes musculus* were found in 36.8 % (7/19) of young mice. Treatment against *Myobia muscili* was more efficient in young than in adult mice ($P < 0.001$); among adult mice, after the second and third weeks of therapy, 36.8 % and

Table 1: The number and percentage of positive samples in relation to sampling techniques

Sampling technique	No. of positive (percentage)*	
	<i>Myocoptes musculinus</i>	<i>Myobia musculi</i>
Fur pluck technique	59 (100.00)**	37 (100.00)**
Sticky paper technique on the back	55 (93.22)	5 (13.51)
Sticky paper technique on the abdomen	54 (91.53)	4 (10.81)

* No. (animals) = 59; samples were taken before the treatment.

** Fur pluck technique was established as gold standard.

Table 2: The number and percentage of positive samples in relation to parasite development forms and sampling techniques

Sampling technique	No. of positive (percentage)*							
	<i>Myocoptes musculinus</i>				<i>Myobia musculi</i>			
	All forms	Eggs	Adults, nymphs and larvae	N-infected (%)**	All forms	Eggs	Adults, nymphs and larvae	N-infected (%)**
Fur pluck technique	17 (28.8)	42 (71.2)	0 (0.0)	59 (100.0)	7 (11.9)	29 (49.2)	1 (1.7)	37 (62.7)
Sticky paper technique on the back	3 (5.1)	50 (84.7)	2 (3.4)	55 (93.2)	0 (0.0)	5 (8.5)	0 (0.0)	5 (8.5)
Sticky paper technique on the abdomen	1 (1.7)	52 (88.1)	1 (1.7)	54 (91.5)	0 (0.0)	4 (6.8)	0 (0.0)	4 (6.8)

* No. (animals) = 59; samples were taken before the treatment.

** Present both species of mites or single infestation with *Myocoptes musculinus*.

Table 3: The number and percentage of positive samples before and after the treatment in all, adult and young mice

Time of collection		No. of positive (percentage)			N (%) – infected*	N –all
		Mixed infestation – <i>Myocoptes musculinus</i> and <i>Myobia musculi</i>	Single infection – <i>Myocoptes musculinus</i>			
All mice	Before treatment	37 (62.7)	22 (37.3)		59 (100.0)	59
	1 st week	34 (58.6)	23 (39.7)		57 (98.3)	58**
	2 nd week	14 (24.3)	29 (50.9)		43 (75.4)	57***
	3 th week	20 (35.1)	25 (43.8)		45 (78.9)	57
Adult mice	Before treatment	31 (79.5)	8 (20.5)		39 (100.0)	39
	1 st week	30 (76.9)	9 (23.1)		39 (100.00)	39
	2 nd week	14 (36.8)	23 (60.5)		37 (97.3)	38**
	3 th week	20 (52.6)	18 (47.4)		38 (100.0)	38
Young mice	Before treatment	6 (30.0)	14 (70.0)		20 (100.0)	20
	1 st week	4 (21.0)	14 (73.7)		18 (94.7)	19***
	2 nd week	0 (0.0)	6 (31.6)		6 (31.6)	19
	3 th week	0 (0.0)	7 (36.8)		7 (36.8)	19

* Present both species of mites or single infestation with *Myocoptes musculinus*.

** One adult mouse died

*** One young mouse died

52.6 %, respectively, were still infected, whereas no infected animals were observed in the group of young animals.

The number of mice infested with adult parasites and their development forms (including eggs) is also shown (Table 4). Using the fur pluck technique before the treatment, the eggs of *Myocoptes musculus* were found on all 59 (100.0 %) mice: in 71.2 % of mice, only eggs were found; in 28.8% of mice eggs, adults, nymphs and larvae were observed. Among 37 mice (62.7 %) infested with *Myobia muscili*, 78.4 % (29/37) had only eggs, 2.7 % (1/37) had only adults, nymphs and larvae, and 18.9 % (7/37) mice had all forms. After the third treatment, 45 (78.9 %) mice were infested for *Myocoptes musculus*. Among them, 82.2 % (37/45) had only eggs, 2.2 % (1/45) had only adults, nymphs and larvae, and 15.6 % (7/45) had all forms. For *Myobia muscili*, 20 (35.1 %) mice were positive and in all (100.0 %) only eggs were found. Almost all eggs of *Myocoptes musculus* and *Myobia muscili* were drained and damaged after second treatment.

Discussion

Evaluation and the usefulness of different sampling techniques

Multiple diagnostic modalities exist. Optimal testing methods, target colony populations, or sampling sites are recommended (3). Fur pluck and sticky paper techniques for the diagnosis of *Myocoptes musculus* and *Myobia muscili* in naturally infested mice were diagnostically evaluated (Table 1). Results before treatment show lower sensitivities of sticky paper techniques on the back and abdomen for *Myocoptes musculus* (93.22 and 91.53 %, respectively) and *Myobia muscili* (13.51 % and 10.81 %, respectively) in comparison with the fur pluck technique. For this reason, the fur pluck technique was chosen as a gold standard for diagnosis during the treatment.

Using the fur pluck technique, we confirmed the presence of eggs of *Myocoptes musculus* in all animals and in 89.8% of them by using both sticky paper techniques. With the use of sticky paper techniques, many false negative results for detecting adults and their development forms of *Myocoptes musculus* were found. Only 8.5 and 3.4 % of animals were found to be positive for adults and their development forms by using the

sticky paper technique on the back and abdomen, respectively, in comparison to the 28.8 % of those forms found with fur pluck technique.

From all mice, 62.7 % were positive for *Myobia muscili* according to the results from the fur pluck technique; eggs were found in 61.1 %, whereas adults and their development forms in 13.6 %. Only 8.5 and 6.8 % of mice were positive (only eggs were found in both cases) according to the results from the sticky paper techniques on the back and abdomen, respectively, showing a high level of false negative results when using sticky paper techniques for diagnosing infection with *Myobia muscili*.

We have established that sticky paper techniques are more suitable for sampling *Myocoptes musculus* than *Myobia muscili*. Sticky paper techniques do not capture all *Myobia muscili* parasites, because of the way mites place eggs on hair. We have observed that *Myobia muscili* place eggs next to the skin, on the bases of two or more hairs together; consequently, the sampling is not always satisfying.

Hairs start to intertwine with each other in dense aggregations. Eggs are larger and heavier, lined along the base of the coat, close to the skin. They are lined along the thinner hairs or mostly on two thinner hairs, so it is more difficult to pick them up with glue. This is the main reason for the small percentage of positive mice for *Myobia muscili* confirmed by sticky paper techniques.

Interestingly, Metcalf Pate et al. (2011) found that sticky paper techniques were more likely to detect fur mites than the fur pluck technique was. They found that the surface temperature of the murine neck surface was warmer than the rump was and thus may represent a unique microenvironment for the development of fur mite eggs. Authors recommend that group-housed adult or preweanling mice should be selected for *Myocoptes musculus* evaluation and that the abdomen should be sampled (3).

The effectiveness of therapy with ivermectin

The treatment of common infestations with mites in conventional colonies of laboratory mice is frequently problematic. The choice of acaricide, the method of application, and the treatment intervals are important in the control of these ectoparasites. Acaricides generally have no effect on acarine eggs. For this reason, re-treatment

after 8–16 days is recommended (4, 9, 14). Wing et al. (1985) concluded that reinfestation must occur if the efficiency of ivermectin ceased before all eggs had hatched (5). In the current study, mice were treated three times at intervals of seven days. Reported ivermectin doses for mice vary from 20 to 400 µg/kg and appear to have been chosen arbitrarily (6). We used ivermectin at a dose 200 µg/kg on BW, similar to the study of Arbona et al. (6, 7).

Ivermectin is one of the most common parasiticides used to treat fur mites (7, 9). It can be administered topically on mice, spraying individual cages of mice, orally in food and water, or by parenteral application administered subcutaneously (4, 6, 7, 11). Davis et al. (15) recorded that ivermectin did not affect the general health, body weight, motor coordination, or spatial learning in several inbred strains of mice. However, it induced a small but significant effect on some sensitive behaviour. At the end of the treatment, we confirmed the presence of *Myobia musculi* eggs in the faeces of one mouse. This proves that mice lick the fur and eat hair, with eggs glued on them and, indirectly, also the drug, which is topically applied on the fur. However, in our study, changes in behaviour were identified neither in adults nor in young mice, and no signs of health problems were observed. The only negative property of ivermectin was a relatively long withdrawal period. It cannot be used on mice intended for the feeding of reptiles for 30 days.

Before treatment, all adult and young mice were infected with *Myocoptes musculinus*, whereas infection with *Myobia musculi* was found in 79.5 % of adults and only in 30.0 % of young mice. Treatment with ivermectin was more effective in young mice. At the end of the treatment period, all examined adult animals were still infected, although mainly drained and damaged eggs were present, whereas only eggs of *Myocoptes musculinus* were found in 36.8 % of young mice. Moreover, with regards to *Myobia musculi*, no infected animal was observed in a group of young animals after the end of the treatment, but 52.6 % of adult mice were still infected (Table 3).

The percentage of infected mice with adults, nymphs and larvae of *Myocoptes musculinus* significantly decreased from 28.8 % before the treatment to 14.0 % after the third treatment ($P=0.043$) and the percentage of mice in which only eggs were found decreased from 71.2 % before

the treatment to 64.9 % after the third treatment without significant difference ($P=0.300$). Based on the results obtained for *Myobia musculi*, we have concluded that ivermectin is effective in the used dosage for adult forms of *Myobia musculi*. A week after the third treatment was completed, only eggs were found (Table 4).

Before and after the treatment, the largest number of mice was infested with eggs. However, after the second application of ivermectin almost all had damaged outer membrane walls. This was regularly observed after the third treatment with ivermectin. In our study, whether eggs were able to survive was not checked. Arbona et al. (2010) assumed that mite eggs and casings can persist for months in mice presumptively treated successfully for fur mites, because eggs are attached permanently to hair shafts and potentially could remain on the animal until the affected hair is shed. They stated that it is extremely difficult to determine whether these mite eggs were viable and whether they had been laid before or after the treatment. Some nonviable eggs could remain until the entire hair coat is shed (16). The presence of mite parts, nonviable eggs, or egg casings, which can remain attached to hair for several months after successful treatment, make it difficult to verify treatment success (7). Techniques for the determination of egg viability were described by Burdett et al. (4, 11). For this reason, it is essential to include confirmation of the vitality of eggs, development forms and adults in routine parasitological diagnostic procedures. We assume that at the end of the third treatment eight mice, in which all forms (7) or adults, nymphs and larvae (1) were, found were still able to infect others with *Myocoptes musculinus*. Therefore, we assume that testing of 59 mice revealed that the efficiency of treatment using this dose of ivermectin for *Myocoptes musculinus* was 86 % effective after three repetitions of treatment.

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ZDRAVLJENJE AKARIAZE Z IVERMEKTINOM IN OCENA RAZLIČNIH METOD VZORČENJA PRI MIŠIH

A. Dovč, R. Lindtner-Knific, I. Markelc, A. Vergles Rataj, M. Gorišek Bajc, P. Zrimšek, M. Pavlak, P. Isaković, K. Vlahović

Povzetek: Vzrejnim mišim, pozitivnim na *Myocoptes musculinus* in *Myobia musculi*, smo akariazo zdravili z ivermektinom v odmerku 200 µg/kg telesne mase (6µl aktivne snovi). Ivermektin smo aplicirali lokalno na kožo v področje zatilja. Aplikacijo smo ponovili trikrat v enotedenskih intervalih. V skupinah odraslih in mladih miši smo pred zdravljenjem, med njim in po njem ugotavljali pristonost različnih razvojnih oblik pršic (odraslih, nimf, ličink in jajčec). Pred zdravljenjem sta bili ovrednoteni dve metodi za zaznavanje različnih oblik pršic: metoda puljenja dlake in metoda lepljenja dlake (vzorčili smo na dveh različnih mestih; na hrbtu in trebuhu). Tehnika puljenja dlake je bila uporabljena kot zlati standard. Občutljivost metode lepljenja dlake je bila 91,5 - 3,2% za *Myocoptes musculinus* in 10,8 - 13,5% za *Myobia musculi*. Ugotovili smo, da *Myobia musculi* odlega jajčeca tik ob koži, na bazi dveh ali več dlak skupaj, zato vzorčenje ni vedno zadovoljivo. Učinkovitost zdravljenja z ivermektinom na *Myocoptes musculinus* in *Myobia musculi* je bila potrjena po drugem zdravljenju. Pred zdravljenjem, med njim in po njem smo opazili značilno višji odstotek pozitivnih miši pri starih živalih v primerjavi z mladimi. Po tretjem zdravljenju smo še vedno ugotavljali tako odrasle kot tudi razvojne oblike (jajčeca, ličinke in nimfe) *Myocoptes musculinus*. Skoraj vsa jajčeca *Myocoptes musculinus* in *Myobia musculi* so bila po tretjem zdravljenju izsušena in poškodovana.

Ključne besede: veterinarska medicina; ektoparaziti; *Myocoptes musculinus*; *Myobia musculi*; zdravljenje