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 Bajc4, Petra Zrimšek4, Marina Pavlak5, Petra Isaković1, Ksenija Vlahović6

1Institute for Health Care of Poultry, 2Institute for Microbiology and Parasitology, 3Clinic for Reproduction and Horses, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, 4Jele 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 musculinus 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 musculinus 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 musculinus 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 musculinus. Almost all eggs of Myocoptes musculinus and Myobia musculi were drained and damaged after the third treatment.

Key words: veterinary science; mice; ectoparasites; Myocoptes musculinus; 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 musculinus, 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 30µl 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 musculinus* 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 musculinus* (Table 1). The sensitivity of the sticky paper technique on the back and abdomen for *Myocoptes musculinus* was 91.5 and 93.2 %, respectively.

Before treatment, 37 (62.7 %) out of 59 examined mice were confirmed to be positive for *Myobia musculi* via the fur pluck technique. *Myobia musculi* 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 musculi*.

Eggs of *Myocoptes musculinus* 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 musculi* 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 musculinus* is statistically verifiable. A significant difference (P<0.05) in the proportion of infected mice after the second (75.4 %) and after the third (78.9 %) week of therapy was found in comparison with the number of infected mice with *Myocoptes musculinus* before treatment (100 %). The effectiveness of therapy against *Myobia musculi* was better than against *Myocoptes musculinus*; after the second and third weeks, only 24.3 and 35.1 % of mice, respectively, were still infected with *Myobia musculi*. The effectiveness of therapy with ivermectin is not evident after the first treatment for *Myocoptes musculinus* (P=0.496), nor is it for *Myobia musculi* (P=0.396). A significant decrease in the number of infected mice with *Myocoptes musculinus* 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 infected with *Myocoptes musculinus* and *Myobia musculi* 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 musculinus* before the treatment, while a significantly lower proportion (P<0.001) of young mice (30.0 %) than adult mice (79.5 %) were infected with *Myobia musculi*. No difference in the proportion of infected mice with *Myocoptes musculinus* between adult and young mice was observed after the first treatment, but after the second and third treatments the proportion of infected 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 musculinus* were found in 36.8 % (7/19) of young mice. Treatment against *Myobia musculi* 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

<table>
<thead>
<tr>
<th>Sampling technique</th>
<th>No. of positive (percentage)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Myocoptes musculinus</em></td>
</tr>
<tr>
<td>Fur pluck technique</td>
<td>59 (100.00)**</td>
</tr>
<tr>
<td>Sticky paper technique on the back</td>
<td>55 (93.22)</td>
</tr>
<tr>
<td>Sticky paper technique on the abdomen</td>
<td>54 (91.53)</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Sampling technique</th>
<th>No. of positive (percentage)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Myocoptes musculinus</em></td>
</tr>
<tr>
<td></td>
<td>All forms</td>
</tr>
<tr>
<td>Fur pluck technique</td>
<td>17 (28.8)</td>
</tr>
<tr>
<td>Sticky paper technique on the back</td>
<td>3 (5.1)</td>
</tr>
<tr>
<td>Sticky paper technique on the abdomen</td>
<td>1 (1.7)</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Time of collection</th>
<th>Mixed infestation – <em>Myocoptes musculinus</em> and <em>Myobia musculi</em></th>
<th>Single infection – <em>Myocoptes musculinus</em></th>
<th>N (%) – infected*</th>
<th>N – all</th>
</tr>
</thead>
<tbody>
<tr>
<td>All mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>37 (62.7)</td>
<td>22 (37.3)</td>
<td>59 (100.0)</td>
<td>59</td>
</tr>
<tr>
<td>1st week</td>
<td>34 (58.6)</td>
<td>23 (39.7)</td>
<td>57 (98.3)</td>
<td>58**</td>
</tr>
<tr>
<td>2nd week</td>
<td>14 (24.3)</td>
<td>29 (50.9)</td>
<td>43 (75.4)</td>
<td>57***</td>
</tr>
<tr>
<td>3rd week</td>
<td>20 (35.1)</td>
<td>25 (43.8)</td>
<td>45 (78.9)</td>
<td>57</td>
</tr>
<tr>
<td>Adult mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>31 (79.5)</td>
<td>8 (20.5)</td>
<td>39 (100.0)</td>
<td>39</td>
</tr>
<tr>
<td>1st week</td>
<td>30 (76.9)</td>
<td>9 (23.1)</td>
<td>39 (100.00)</td>
<td>39</td>
</tr>
<tr>
<td>2nd week</td>
<td>14 (36.8)</td>
<td>23 (60.5)</td>
<td>37 (97.3)</td>
<td>38**</td>
</tr>
<tr>
<td>3rd week</td>
<td>20 (52.6)</td>
<td>18 (47.4)</td>
<td>38 (100.0)</td>
<td>38</td>
</tr>
<tr>
<td>Young mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>6 (30.0)</td>
<td>14 (70.0)</td>
<td>20 (100.0)</td>
<td>20</td>
</tr>
<tr>
<td>1st week</td>
<td>4 (21.0)</td>
<td>14 (73.7)</td>
<td>18 (94.7)</td>
<td>19***</td>
</tr>
<tr>
<td>2nd week</td>
<td>0 (0.0)</td>
<td>6 (31.6)</td>
<td>6 (31.6)</td>
<td>19</td>
</tr>
<tr>
<td>3rd week</td>
<td>0 (0.0)</td>
<td>7 (36.8)</td>
<td>7 (36.8)</td>
<td>19</td>
</tr>
</tbody>
</table>

* 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 musculinus* 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 musculi*, 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 musculinus*. 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 musculi*, 20 (35.1 %) mice were positive and in all (100.0 %) only eggs were found. Almost all eggs of *Myocoptes musculinus* and *Myobia musculi* 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 musculinus* and *Myobia musculi* 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 musculinus* (93.22 and 91.53 %, respectively) and *Myobia musculi* (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 musculinus* 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 musculinus* 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 musculi* 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 musculi*.

We have established that sticky paper techniques are more suitable for sampling *Myocoptes musculinus* than *Myobia musculi*. Sticky paper techniques do not capture all *Myobia musculi* parasites, because of the way mites places eggs on hair. We have 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 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 musculi* 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 musculinus* 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 reinfection 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 administration 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.

References


2. Lindstrom KE, Carbone LG, Kellar DE, Mayorga MS, Wilkerson JD. Soiled bedding sentinels for the detection of fur mites in mice. J Am


ZDRAVLJENJE AKARIAZE Z IVERMEKTINOM IN OCENA RAZLIČNIH METOD VZORČENJA PRI MIŠIH


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