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Influence of topical therapy with the parasiticide Ivermectin on embryo transfer in mice.

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Introduction

At the time of the study, the animal facilities at the European Molecular Biology Laboratory (EMBL) consisted of a conventional open unit, approximately 20 years old, which was in the process of being closed down, and a new animal house, run as a closed unit meeting European guidelines (Council of Europe, 1986), which was being set up. The old animal house contained all EMBL's transgenic lines together with the old breeding colonies of wild, type mice (inbred strains and outbred stocks) and "stock" animals necessary for the production of new transgenic animals. New breeding colonies were being established in the new animal unit, while the existing transgenic lines from the old animal house were being rederived by embryo transfer into the new unit. Mice housed in the old 5 unit were found to be affected by endoparasites, namely *Syphacia muris* and *S. obvelata* as detected in smear preparations of caecal and colonic contents (BRL, Switzerland). Furthermore the ectoparasite *Myobia musculi,* was diagnosed from microscopic examination of hair and skin (BRL, Füllinsdorf, Switzerland).

Ecto- and endoparasitism can cause welfare problems in mice: acariasis, for example, may cause intense itching resulting in scratching and possible self-trauma, especially in black strains of mice (*Harkness & Wagner,* 1983). Helminth infections, although usually asymptomatic, may cause various intestinal lesions including rectal prolapse, intussusception, enteritis and faecal impaction in heavily infected mice (*Jacoby and Fox*, 1984). Furthermore, reduced growth rate has been reported in infected mice (*Cunliffe-Beamer & Les*, 1987) and reproductive depression (*Harkness & Wagner,* 1983). Therefore affected mice should be treated with a suitable parasiticide to avoid welfare problems but, where experimental mice are involved (as in the present case), the treatment should be chosen with care so that experimental results are not invalidated.

Various parasiticides are available for treating domestic animals but few of these products have been tested specifically for the treatment of rodents and may occasionally be toxic in these species. Furthermore, the route of administration should be considered carefully when selecting the therapy, since ease of application is essential when treating several thousand animals. Organophosphates can be applied easily, either topically or on resin-impregnated strips placed in the cage (*Harkness & Wagner*, 1983). However, these compounds may delay breeding transiently in treated mice *(Jacoby and Fox*, 1984), while accidental exposure of mice was found to have a detrimental effect on the success of embryo transfer studies *(Morrell et al.*, 1995). Ivermectin has been recommended as an effective parasiticide for mite infestation in mice when used as a spray *(Baumans et al.*, 1988a; *Le Blanc et al*, 1993) and to control both oxyurid and mite infections in mice, again as a spray *(Baumans et al.*, 1988b), but potential effects on embryo quality or breeding performance of treated mice were not investigated in these studies.

Since the infestations could be causing welfare problems, both for the animals and staff, it was decided to treat the mice. Ivermectin appeared to be the preferred drug for treatment, because of its ease of application and documented efficacy in mice (*Baumans et al.*, 1988a; *Baumans et al.*, 1988b; *Le Blanc et al*, 1993). A preliminary study was carried out to determine whether exposure of

mice to ivermectin could affect embryo production during superovulation or influence embryo survival and further development in embryo transfer studies. The outcome of this study and of the subsequent treatment of approximately 6000 transgenic mice with ivermectin is described here.

Materials and Methods

Animals and Husbandry

i.study group, new animal house

Thirty adult CD1 females, 8 weeks old, and 40 juvenile C57BL/6 females, 21 days old, from in-house breeding colonies of SPF status were available as embryo and recipients donors respectively. Half the donors and half the recipients were treated with ivermectin, as described later. Ten adult CD1 males were vasectomised prior to the present study and were shown to be sterile in test matings. All mice were housed conventionally in standard cages (B type: Scanbur, Køge, Denmark) provided with aspen bedding and nesting material (Tapvei, Kortteinen, Finland), while tap water and rodent breeding diet (RM3; Special Diet Services, Boxmeer, The Netherlands) were available ad libitum. Access to the animal rooms was strictly limited. The environmental conditions in the unit (21° ± 2° C and relative humidity 55 ± 10%) and the light:dark cycle (12 hour light, 12 hour dark) were monitored continuously by computer.

ii. transgenic and breeding colonies, old animal unit

Transgenic mice of different ages and with a variety of genetic backgrounds, and the BL6 stud males required for mating the superovulated embryo donors, were housed conventionally in the old animal unit at EMBL. Acidified tap water (0.005% hydrochloric acid) and non-irradiated mouse maintenance diet (Herilan rat, mouse, hamster food; Eggersmann Futtermittelwerk, Rinteln, Germany) were available *ad libitum*. Wood shavings (Einstreu; Rueckmann, Leimen, Germany) were provided as bedding material.

Treatment with Ivermectin.

A solution of ivermectin was prepared by diluting Ivomec 1% (Merck, Sharpe & Dohme AGVET, Hoddesdon, UK) 1:100 with a mixture (1:1 v/v) of tap water and propylene glycol (Sigma Chemical Co., Deisenhofen, Germany). A fine spray of ivermectin was delivered from a proprietary plant spray bottle over the inhabitants of the cage, ensuring that each animal received some of the spray, as ascertained by the appearance of moisture on their coats.

The same formulation and method of administration of ivermectin were used to treat the main colony. Nursing mothers were removed from the cage for spraying so that the babies were not directly treated.

Embryo collection and freezing

Juvenile females C57BL/6 bred at EMBL, were superovulated using standard techniques (Hogan et al., 1986) and mated to males of known fertility which were kept in a separate animal unit housing the transgenic colony. Briefly, the superovulation procedure involved administering 5 IU follicle stimulating hormone by intraperitoneal injection, followed 47 hours later by 5 IU human chorionic gonadotrophin, also by intraperitoneal injection. Embryos were recovered from the reproductive tract of the donors by bursting the swollen ampullary region of the oviduct in a drop of medium (*Hogan et al.*, 1986). They were assessed subjectively for quality; only those embryos which were considered to be normal in appearance (i.e. having a rounded smooth appearance with an intact zona pellucida and homogeneous non-granular cytoplasm evenly distributed within the cytoplasmic membrane, subjectively classified as "good") were used for transfer.

Embryo transfer.

The scheme for the transfers was based on a 2x2 contingency table with the following "treatment" groups: embryos from treated mice transferred into treated recipients, embryos from untreated mice transferred into treated recipients, embryos from treated mice transferred into untreated recipients, embryos from untreated mice transferred into untreated recipients.

Pseudopregnant CD1 females (day 0.5 or 1.5), obtained by mating females to vasectomised males, were anaesthetised with tribromethanol. Six or seven embryos, depending on the number avai-

lable, were transferred into each oviduct using aseptic procedures (modified from Hogan et al., 1986). Briefly, each ovary and oviduct exposed through a lateral laparotomy incision. The ovarian bursal membrane was incised to provide access to the infundibulum for the transfer pipette containing the embryos. After delivery of the embryos, the peritoneum, muscle and skin layers were each sutured and the mice were kept warm until recovery from anaesthesia was complete. Skin sutures were removed after 7 days. At 15 days after transfer, the number of foetuses and resorption sites present in the uterus were counted.

The number of treated and untreated recipients available on each day was not equal, being dependent on the number of mice mating each night, with the result that the "treatment groups" contained different numbers of recipients.

Results

Preliminary study

Application of ivermectin spray caused an immediate increase in grooming activity to the exclusion of other activities, with all animals participating for approximately 5 minutes. After this time, normal activities such as exploring or eating, gradually resumed. No long term effects of the spraying, for example prolonged excessive grooming, or clinical signs such as diarrhoea, alopecia or death were seen in the treated group.

Overall, in both treated and untreated mice, the rate of plugging of superovulated mice was lower than expected for our mice (45% compared to approximately 80%). There were slight differences between the number of donor mice plugging, yield of embryos and proportion of good embryos between the two groups (Table 1), with fewer mice plugging from the treated group and fewer embryos being produced, but these differences were not statistically significant (Student's test).

The number of implantations and resorptions for each treatment group are shown in Table 2 while the proportion of the embryos transferred which implanted or resorbed for each combination of donor and recipient are shown in Figure 1. The differences in implantation and resorption rates between groups are not significant using the Chi squared test on a 2x2 contingency table.

Treatment of main colony.

No problems with reproductive efficiency were observed in naturally mated mice after treatment of the main colony with ivermectin. No clinical signs of diarrhoea appeared after treatment and no mismothering of litters was reported. Ecto- and endoparasites were absent from all animals (n = 30) at the next routine health screen, which took place approximately 4 weeks later (Prof. J.R. Needham; The Microbiology Laboratories, London, UK), following FELASA recommendations (Kraft et al., 1994). The diagnostic tests used were microscopic examination of the skin and hair (ectoparasites) and microscopic examination of a wet preparation of the intestinal contents (Needham, 1979).

Table 1: Recovery of embryos from treated and untreated mice.

	Treated	Untreated	Comments
No. mated	20	20	
No. plugged	8	10	NS
No of embryos	277	388	
No. of embryos/plug *	34.6 ± 4.3	38.8 ± 2.7	NS
No. good embryos	203	271	
No. good embryos/plug *	25.4 ± 5.8	27.1 ± 2.5	NS

Note: NS = difference is not significant (t test) *** = mean ± SE**

Table 2: Implantation and resorption rates of embryos from treated and untreated donors transferred into treated and untreated recipients.

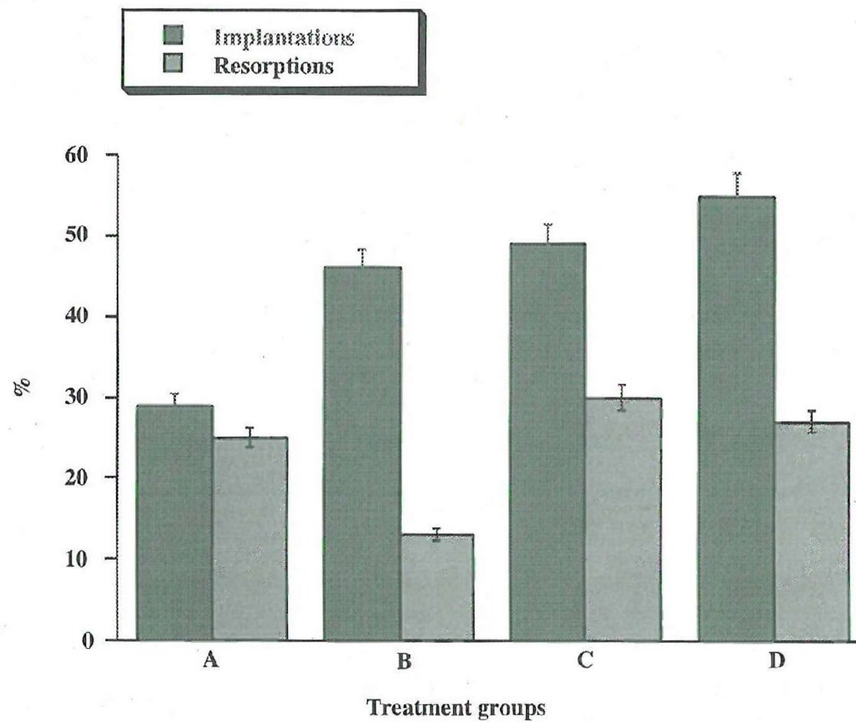
	TDi	UDi	TDr	UDr
TR	29	46	86	30
UR	49	55	62	49

Note: TR = treated recipients, UR = untreated recipients, TD = treated donors, UD = untreated donors.

$$\text{Implantation rate (i)} = \frac{\text{number of embryos implanting}}{\text{number of embryos transferred}}$$

$$\text{Resorption rate (r)} = \frac{\text{number of embryos resorbed}}{\text{number of embryos implanted}}$$

Figure 1. Number of embryos implanted and resorbed as a proportion of the total number of embryos transferred for each treatment group. A = treated recipients, treated donors; B = treated recipients, untreated donors; C = untreated recipients, treated donors; D = untreated recipients, untreated donors.



Discussion

In the preliminary study, ivermectin did not appear to cause long term clinical effects on treated mice. There was no statistical difference in the rate of plugging, number of embryos produced, implantation rate or resorption rate between treatment and control groups. This result was encouraging since it appeared to indicate that ivermectin could be used as a parasiticide for the transgenic colony without causing untoward side-effects which might interfere with experimental work. The reason for the reduced plugging rate of all superovulated animals in this study compared to the expected rate for our colony remains uclear.

The use of ivermectin as a spray facilitated the treatment of large numbers of mice in the main colony. It was relatively easy to ensure that all mice were treated because the droplets could be seen wetting the hair coat, in contrast to administering the drug in the drinking water or adding it to feed when the dose received depends on individual intake. Furthermore direct application was more cost effective than inclusion in the diet or drinking water. No ill-effects in terms of decreased reproductive efficiency, mis-mothering or clinical diarrhoea were observed when the main colony was treated, nor did babies appear to suffer any ill-effects from the administration of the drug to their mothers.

Therefore it would appear that iver mectin can be used as described here on mice which are to take part in embryo transfer studies without detrimental effects on implantation or resorption rates.

Summary

It was necessary to treat a colony of several thousand transgenic and wild, type breeding mice for parasitic infestations. The suitability of the anti-parasitic agent, ivermectin, for controlling parasites in mice which are to be used for embryo transfer was investigated in a small preliminary study. There were no significant differences in plugging rate, number of embryos produced, number of implantations or number of resorptions between treated and untreated mice. Therefore the main colony of transgenic mice was treated with ivermectin to remove oxyurid worms and mites. The treatment was effective, since no parasites were found at a subsequent health screen, and there were no adverse effects such as diarrhoea or mis-mothering in treated mice. These results indicate that ivermectin could be used in mice for transgenic studies without causing detrimental effects on either the mice or the experiment.

References

Baumans, V., R. Havenaar & H. van Herck: The use of repeated treatment with Ivomec and Neguvon spray in the control of murine fur mites and oxyurid worms. Lab. Anim. 1988, *22*, 246- 249.

Baumans, V., R. Havenaar & H. van Herck: The effectiveness of Ivomec and Neguvon in the control of murine mites. Lab. Anim. 1988, 22, 243-245.

Council of Europe: European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Procedures. HMSO, London, 1986.

Cunliffe-Beamer, T. & E. Les: The laboratory mouse. Ch.18 in The UFAW Handbook on the Care and Management of Laboratory Animals. T. Poole (ed.) 6th edn. Longman Scientific & Technical, Harlow, 1987, pp 275-308.

Le Blanc, S., R. Faith & C. Montgomery: Use of topical ivermectin treatment for Syphacia obvelata in mice. Lab Anim. Sci. 1993, *43*, 526-528.

Harkness, J. & J. Wagner: Biology and Medicine of Rabbits and Rodents. 3rd edn. Lea & Febiger, London, 1983.

Jacoby, R. & J. Fox: Biology and diseases of mice. Ch. 3 in Laboratory animal medicine. Fox, J., Cohen, B. & Loew, F. (eds.) Academic Press Inc, London, 1984, pp 31-91.

Kraft, V., A. Deeney, H. Blanchet, R. Boot, A Hansen, A. Hem, H. van Herck,I. Kunstyr, G. Milite, J. Needham, W. Nicklas, A. Perrot, C. Rehbinder, Y. Richard & G. De Vreoy: Recommendations for Health monitoring of mouse, rat, hamster, guinea pig and rabbit breeding colonies, Lab. Anim. 1994, *28*, 1-12.

Morrell, J., M. Gore & P. Nayudu: Retrospective observation of the effect of accidental exposure to organophosphates on the success of embryo transfer in mice. Lab Anim. Sci. 1995, *45*, 437-440.

Needham, J.R.: Handbook of microbiological investigations for laboratory animal health. Academic Press Inc., London, 1979.