A Laboratory Animal Model of Mare Reproductive Loss Syndrome: Preliminary Evaluation of a Mouse Model

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DURING 2001, CENTRAL KENTUCKY HAD AN EPIDEMIC OF early and late fetal losses (EFL/LFL) that was together called Mare Reproductive Loss Syndrome (MRLS). The LFL began in the last week of April, peaked on May 5, and declined rapidly. EFL was identified on April 26 and had a similar course and ultimately totaled about 1,500 cases (1). The same syndrome was repeated in May and June of 2002 with fewer losses. Concurrent with each epidemic was a local population explosion of eastern tent caterpillars (ETC), *Malacosoma americanum*, with large numbers wandering in pastures. Epidemiological studies, period of occurrence, and experiments conducted during the last year strongly suggest that exposure to ETC plays an important role in this syndrome.

Because of the size, long gestation period, and expense of pregnant mares, a laboratory animal was needed for study of this syndrome. It was hoped that abortions could be induced in mice by exposing them to the products of ETC. A study was undertaken to determine if ETC, frass (droppings of caterpillars), and setae (hairs) administered through different routes could produce abortion and, if so, to study the toxicopathologic effect of ETC.

Materials and Methods

Four challenge experiments with pregnant mice (ICR, Taconic Labs, Germantown, NY) were performed with ETC, frass, and setae administered by various routes. Caterpillars and frass were weighed and mixed in normal saline with a mortar and pestle. The solutions were transferred to a tissue homogenizer and finely homogenized with the volume adjusted to 0.5 ml/mouse. The homogenates were administered by gavage using a ball-tipped needle (Perfekturn, Popper & Sons Inc., New Hyde Park, NY). Setae (15 setae/mouse) were plucked from the skin of caterpillars and homogenized in a tissue homogenizer with normal saline, and the volumes were adjusted to 0.4 ml/mouse. All materials were prepared fresh daily for administration.

In Experiment 1, three groups of mice (12 days pregnant) were administered frass (19 mg; n = 9) that had been frozen, early instar ETC (70 mg; n = 9) that had been frozen, and saline (0.5 ml; n = 8) by oral gavage. The experiment was terminated on day 19 of pregnancy when the mice began to give birth. All mice and pups were euthanized, and complete necropsies were performed.

In Experiment 2, fresh frass and ETC were used. Three groups of mice (12 days pregnant) were administered fresh frass (19 mg; n = 7), late instar ETC (200 mg; n = 7), and saline (0.5 ml; n = 7) by oral gavage. The experiment was terminated on day 18 of pregnancy. All animals were euthanized, and full necropsies were performed.

In Experiment 3, two control groups of mice (5 days pregnant) were administered saline by oral gavage (0.5 ml; n = 4) and saline by intraperitoneal (IP) injection (0.4 ml; n = 4), and three treatment groups (5 days pregnant) were dosed for 14 days with one of the following: fresh frass (19 mg; n = 7) by gavage, late instar ETC (200 mg; n = 8) fed on fresh cherry tree leaves by gavage, or setae plucked from live late instar caterpillars filtered through a bacterial filter (VWR Scientific Products, West Chester, PA) by IP injection (20 setae/mouse; n = 7). The reason for injecting setae was to evaluate the possible role of a soluble setal toxin in MRLS. However, because of a personnel change occurring 4 days into this experiment, all later setal homogenate injections were unfiltered. This experiment can therefore be interpreted only in terms of a setal homogenate effect. The experiment was terminated on the nineteenth day of pregnancy. All animals were euthanized, and full necropsies were done.

In Experiment 4, three groups of mice (5 days pregnant) were treated by IP injection for 14 days. Groups were administered one of the following treatments: saline (0.4 ml; n = 7) to a control group, a filtered homogenate/extract (20 setae/mouse; n = 7), and an unfiltered homogenate/extract (20 setae/mouse; n = 7). Setae were plucked from frozen late instar caterpillars, and the homogenate was filtered through a bacterial filter. The experiment was terminated by euthanasia on the eighteenth day of pregnancy, and full necropsies were performed. Both filtered and unfiltered homogenates were injected to distinguish between a setal toxin (filtered homogenate) and mechanical irritation or bacterial-laden setae disrupting the fetal membranes (unfiltered homogenate).

Results

The gross and histopathological findings of the four experiments are detailed in Tables 1 through 4. In Experiment 1, there were no significant findings in the uteri of

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the mice that died during the experiment. Furthermore, there were no significant findings in the uteri and fetuses of any of the mice in treatment and control groups. The total number of pups born for the treatment and control group mice are shown in Table 1.

In Experiment 2, the uterus of the mouse that reabsorbed one fetus had acute suppurative inflammation at the implantation site. There were no significant findings in the uteri (implantation sites) and fetuses of any mice in the treatment and control groups. The total number of pups in the uteri of treatment and control mice are shown in Table 2.

In Experiment 3, the group administered setae had statistically significant reabsorptions of all fetuses in three mice, and two mice were not pregnant. The uteri of the mice that had reabsorptions had acute suppurative inflammation at the implantation sites. The total number of pups in the uteri of treatment and control mice and the histopathological findings in the uteri of setae-administered mice are shown in Table 3.

In Experiment 4, two fetuses were reabsorbed in the unfiltered setae group, eight fetuses were reabsorbed in the filtered group, and four fetuses were reabsorbed in the control group. The uteri of the mice that had reabsorption showed acute suppurative inflammation at the placental sites. The total number of pups in the uteri of the treatment and control group mice are shown in Table 4. The bacteria isolated from the uteri of mice in Experiment 1, 3, and 4 are detailed in Table 5.

Table 1. Number of pups for each mouse in Experiment 1. Group 1 was administered caterpillar extract (early instar), Group 2 was administered frass, and Group 3 (control) was administered normal saline.

Animal ID	No. of Pups	Comment
Grp1-1	14	DEAD - during experiment
Grp1-2	5	
Grp1-3		No pups born when euthanized - 10 at necropsy
Grp1-4	13	DEAD - during experiment
Grp1-5	11	
Grp1-6	14	
Grp1-7	12	
Grp1-8	14	
Grp1-9	12	one not active
Grp2-1	7	pups not active
Grp2-2	13	DEAD - during experiment
Grp2-3	11	
Grp2-4	12	
Grp2-5	11	DEAD - during experiment
Grp2-6		No pups born when euthanized - 11 at necropsy
Grp2-7	12	
Grp2-8	14	
Grp2-9	11	
Grp3-1	13	
Grp3-2	10	
Grp3-3	13	
Grp3-4	9	
Grp3-5	11	
Grp3-6	14	DEAD - during experiment
Grp3-7	10	
Grp3-8	12	

Discussion

In Experiment 3 (setae from live ETC), three of the five pregnant mice had reabsorption of their fetuses. The same experiment, when repeated with frozen setae (Experiment 4), did not produce reabsorption as observed in Experiment 3. The structure and composition of setae, whether it changes by freezing and subsequent thawing, are not known. Normally 10 to 20% of reabsorption is noticed in the uteri of pregnant mice. Reabsorption in the control groups of Experiments 2, 3, and 4 was not above 10%, and no significant pathological changes were observed. In Experiments 1, 2, and 4, the percentage of reabsorptions was also below 10% in the treatment groups.

It was hoped that the mouse would mimic MRLS seen in horses so that a laboratory animal model could be used to more thoroughly investigate MRLS. Horses aborted following oral dosing with 50 g ETC to a 500-kg horse (0.1 g/kg). The mice did not abort following oral dosing with 200 mg ETC to a 20-g mouse (10 g/kg). So even though mice received a 100-fold increase in ETC per body weight, they were not susceptible to abortion. The reason(s) for the difference(s) in susceptibility of the two animals could be different intestinal enzymes and/or flora, different placentation, or longer gastrointestinal tract in the horse increasing the possibility of intestinal absorption. The intestinal tract appears to be involved because, when it was bypassed with IP injections of setal homogenate/extract from live ETC, mouse reabsorptions did occur in three of the five mice.

There also appears to be a factor associated with setae from live ETC versus frozen ETC. Since Experiment 4 was run after the supply of live ETC had been exhausted, setae were taken from frozen ETC. There were no effects from IP injection of setal extract in that experiment.

Conclusion

Pregnant mice are not as susceptible to ETC-induced abortion as horses. Mice do not produce abortions where the expelled fetuses and placentas can be examined, but rather mice reabsorb the fetuses. The gross and histopathological changes in the experiments conclude that mice (*Mus musculus*) are not a suitable laboratory animal for reproducing MRLS.

Reference

1. Harrison, L. R. Kentucky equine abortion storm and related conditions. Proceedings of the United States Animal Health Association. 2001; (105): 227-229.

Table 2. Number of pups for each mouse in Experiment 2. Group 1 was administered fresh frass, Group 2 was administered fresh ETC, and Group 3 (control) was administered normal saline.

Animal ID	No. of Pups	Comment
Grp1-1	7	
Grp1-2	12	
Grp1-3	13	
Grp1-4	2	
Grp1-5	13	
Grp1-6	10	
Grp1-7	14	
Grp2-1	12	
Grp2-2	11	
Grp2-3	14	
Grp2-4	15	
Grp2-5	10	
Grp2-6	13	
Grp2-7	12	
Grp3-1	14	
Grp3-2	12	one fetus reabsorbed
Grp3-3	10	
Grp3-4	13	
Grp3-5	13	
Grp3-6	13	
Grp3-7	10	

Table 3. Pups for each mouse in the uteri of setae group of Experiment 3.*

Animal ID	Total No. of Fetuses	Reabsorption	Comment
Control 1	10		
Control 2	4		
Control 3	11	One dead fetus	
Control 4	12		
Setae 1	11		
Setae 2	11		
Setae 3		All fetuses reabsorbed	Acute suppurative inflammation at implantation site
Setae 4			Not pregnant
Setae 5			Not pregnant
Setae 6		All fetuses reabsorbed	Acute suppurative inflammation at implantation site
Setae 7		All fetuses reabsorbed	Acute suppurative inflammation at implantation site
Caterpillar group	No abnormalities noticed in the uterus and fetuses		
Frass group	No abnormalities noticed in the uterus and fetuses		
Control group-2	No abnormalities noticed in the uterus and fetuses		

* The histopathological findings of uteri of mice that had reabsorptions are listed in column 3.

Table 4. Number of pups for each mouse in Experiment 4. Group 1 received unfiltered setae, Group 2 received filtered setae, and Group 3 (control) received normal saline.

Animal ID	Pups Born	Comment
Grp1-1	1	
Grp1-2	2	Left horn thickened
Grp1-3	13	
Grp1-4	Non-pregnant	
Grp1-5	11	Normal
Grp1-6	12	One reabsorbed
Grp1-7	3 dead, 3 live	Had pups before euthanasia
Grp1-8	5 dead	Had pups before euthanasia 1 small fetus
Grp1-9	7 live, 2 dead	Had pups before euthanasia
Grp1-10	9	1 reabsorbed
Grp2-1	15	3 reabsorbed
Grp2-2	12	normal
Grp2-3	Non-pregnant	
Grp2-4	Non-pregnant	
Grp2-5	11	
Grp2-6	10	
Grp2-7	5	4 reabsorbed
Grp2-8	Non-pregnant	
Grp2-9	5 live, 2 dead	Had pups before euthanasia
Grp2-10	12	1 reabsorbed
Grp3-1	7	Had pups before euthanasia
Grp3-2	1	
Grp3-3	Non-pregnant	
Grp3-4	12	
Grp3-5	11	1 reabsorbed
Grp3-6	12	1 reabsorbed
Grp3-7	9	
Grp3-8	12	Had pups before euthanasia
Grp3-9	14	1 reabsorbed
Grp3-10	13	1 reabsorbed

Table 5. Bacteria isolated from Experiments 1, 3, and 4 and from one of the filters used to filter setae.

Experiment 1	Experiment 3	Experiment 4	Filter
No growth	*Serratia marcescens* - setae group	*Serratia marcescens*	*Pseudomonas maltophila*
	Pantoea agglomerans - setae group	*Pseudomonas maltophila*	Unclassified gram negative bacillus