Elimination of mouse hepatitis virus (MHV) from a breeding colony using serological testing and isolation

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Introduction
The laboratory mouse with its numerous strains is by far the commonest laboratory animal in use today (1). Published surveys suggest HMV is a common infection present in breeding populations of mice (2, 3). Current personal experience at the University of Cambridge supports this. A wide range of effects have been observed in mice as a result of MHV infection (4, 5). These effects include alteration of immune function, variation in tumour growth and survival, altered hepatic function, anaemia, thrombocytepenia, increased susceptibility to other mouse pathogens, overt clinical disease and death. In addition, it may also contaminate cell lines and interfere with the recovery of other viruses. MHV has therefore a great potential for disrupting research.
It is fortunate that the physical properties of the virus, the epizootiology of infection, and the ready availability of sensitive serological tests, has allowed a number of methods for elimination of the virus from mouse colonies to be developed.
A colony of Peruvian mice carrying the C6QO gene had been established at the Animal Holding and Breeding Unit at the University of Cambridge. This colony was being used to transfer the C6QO gene to a C3H/He background by backcrossing. The backcrossing and testing scheme is shown in Diagram 1. During this period, the original Peruvian colony and backcrosses had become infected with MHV. For managemental reason, it became necessary to eliminate this foci of infection. A number of alternatives were examined and rejected for reasons of practicality before the final method was chosen.
The Peruvian mice were being used for test mating to identify backcross mice heterozygous for the C6QO gene. It was decided at the time, again for reasons of practicality, not to attempt to eliminate MHV from the Peruvians, as the colony itself could be culled once a clean group of homozygous backcrosses was established.

Materials and methods
Animals
10 male and 10 female mice of the latest C3H/He backcross generation were selected from 10 litters of males known to be C6QO heterozygotes. Identifying those animals selected as positively carrying the C6QO gene was not possible. This would have involved test mating with the only C6QO homozygotes available at the time which were the Peruvians and testing the offspring. As the Peruvian mice are poor breeders, this would have resulted in an unacceptable time delay. Direct testing for C6 was not possible as the C6 blood levels in C6QO heterozygotes do not vary sufficiently from the normal animal to be detected by the tests then available. Using a test for C7, a close linked gene increased the chances of the animals selected being C6QO heterozygotes. The test is based on C7 serum enzyme polymorphism. This test was only possible on the male animals as the C7 blood levels are insufficient in the female for the sensitivity of the test to distinguish. Six pathogen free MF1 outbred mice were selected as sentinel animals.

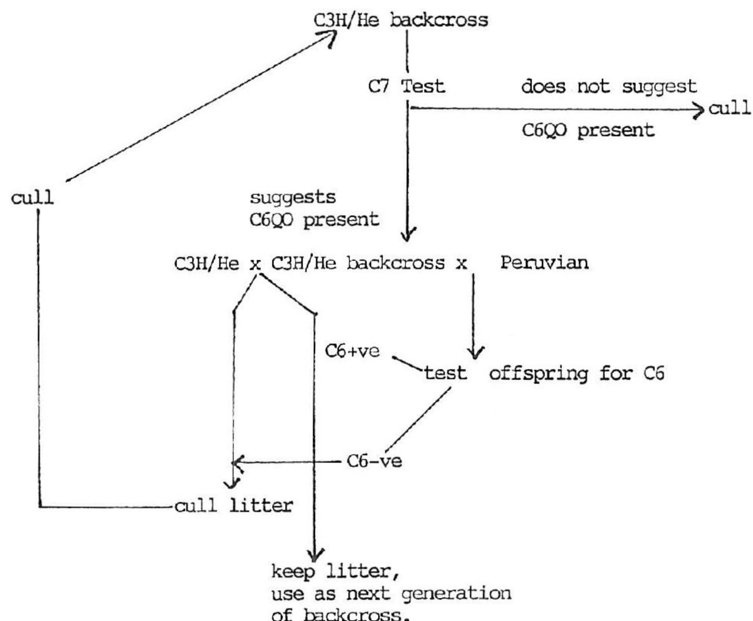

Diagram 1. Original Breeding Scheme for Backcrossing and Testing.

Serology

Blood samples were sent to the Royal Veterinary College, London to be tested for antibodies to MHV using an ELISA test. Samples were obtained from live animals by tail bleeding under ether anaesthesia into heparinised capillary tubes. Three fifty microlitre capillary tubes were filled from each mouse. The plasma was separated off using a microhematocrit centrifuge. The capillary tubes were then broken at the cell-plasma junction, and the plasma expressed into a collection pot.

Housing and Transfer Facilities

The selected C3H/He backcrosses were housed in a standard Isotec positive pressure isolator. The sentinel animals were housed in a separate Isotec positive pressure mini isolator. Servicing of these modules was by standard methods. A standard caesarian rederivation unit was used for transfer of the C3H/He backcrosses into the isolator. The only essential part of the latter was the disinfectant dunk bath. The remainder of the colony was kept in a separate room within the same facility.

Environment

During the course of the exercise, temperatures ranged between 18°C and 28°C, and relative humidity ranged between 29 % and 70 %. The photoperiod was controlled by an automatic time switch giving 12 hours light and 12 hours dark. The mice were housed in standard North Kent Plastic small cages with Biotech grade 2/2 wood chip bedding. Biosure CRM irradiated diet and tap water were supplied ad libitum to the mice housed in the isolator. BKRM diet and tap water were supplied ad libitum to mice housed in the main room.

Method

The selected backcross mice were bled, and the samples tested for antibodies to MHV. Those mice that were positive could be used for the procedure. The MF1 sentinel mice

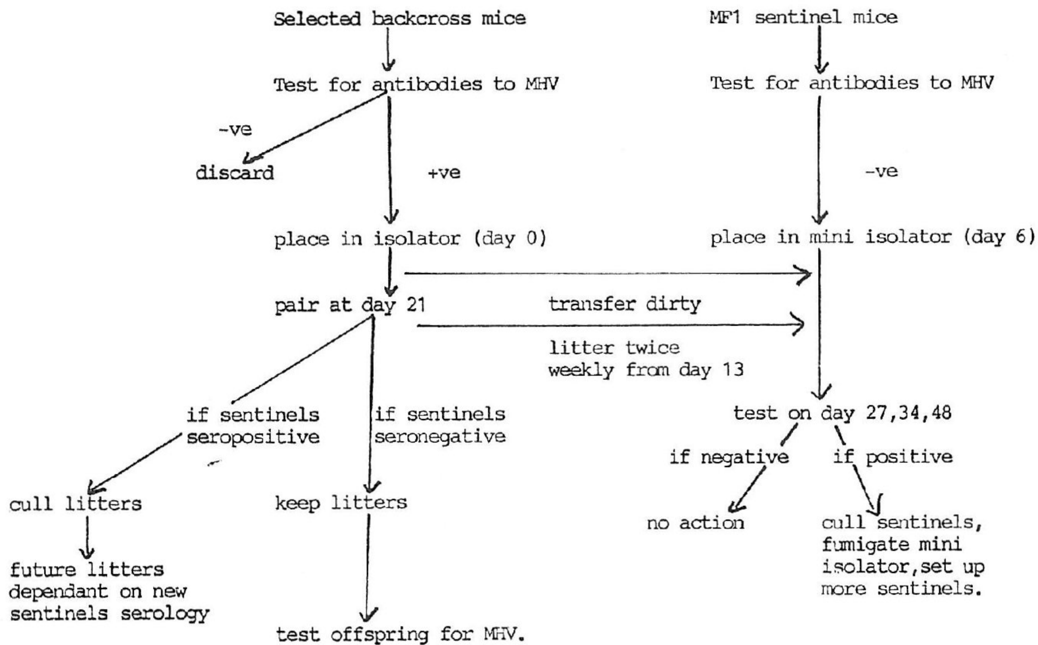

Diagram 2.

were bled on arrival to ensure that they were MHV antibody negative.

The isolators were equipped and fumigated using formaldehyde by standard methods. The caesarian redivation unit was connected to the main isolator. The dunk bath was filled with 1 % Virkon and the 'clean' side of the unit misted with 1 % Virkon. Three people were required during the transfer. The first person placed each cage in the dirty side of the unit, and then placed the mice one by one into the dunk bath passing the tail of the animal under the central barrier. The tail was then grasped by the second person working on the clean side of the unit, and the mouse pulled through. This resulted in brief submersion (about one second) with thorough wetting of the animal coat.

The animal was then caged and the cage passed through to the person operating the isolator. When all mice were transferred, the inner port was closed and the unit removed. The day of transfer is day 0.

The mice were housed in eight cages, four containing males and four containing females. The cages were cleaned twice weekly. The dirty litter was mixed together, and then used for bedding the sentinel mice on a 50:50 mixture of dirty with clean litter. The sentinel mice were placed in the mini isolator on day 6. Dirty litter transfer began on day 13.

Blood samples for MHV testing were taken from the sentinel mice on the following days: 27, 34, 48 and 76. The sentinels were killed at the time of the last sampling. The backcross mice were due to be paired up on day 21, and the first litters produced 21 days after that. If at any time the sentinel mice became positive, it was decided that the backcross mice would have the pairing delayed, or if this had already occurred, any young produced would be killed. The sentinel mice would also be killed, the mini isolator refumigated, fresh sentinels obtained and the litter transfer and blood testing re-

sumed. No young would be kept until the new sentinels had been shown to have remained seronegative for 3 weeks. Offspring produced by the backcrosses were tested at 8–9 weeks of age to check seroconversion had not occurred. Animals from the main room of a similar age were also taken for serology at the same time to demonstrate a continuing pressure of MHV.
The general scheme is shown in Diagram 2.

Results
The results of the serological testing are presented in Table 1. As can be seen, all those backcross mice initially selected were positive for antibodies to MHV except for one, and hence all but this one could be used for the procedure. At no time did the sentinel mice seroconvert. 8–9 week old offspring of the backcrosses were serologically negative for MHV. 9–10 week old animals taken from the main room were serologically positive for MHV.

Table 1. MHV Serology Test Results.

Day	Animals	Serology result		
		Positive	Weak Positive	Negative
0	Backcrosses	10	9	1
6	MF1 Sentinels	–	–	6
27	,,	–	–	6
34	,,	–	–	6
48	,,	–	–	6
76	,,	–	–	6
105	Isolator off-spring	–	–	5
105	Open mouse room	6	–	–

Discussion
A number of different procedures have been described as a means of eliminating MHV infection i.e. caesarian rederivation (6), embryo transfer (7), cross fostering using microisolators (8), temporary cessation of breeding (9). Each of these had practical problems which prevented them from being used in this instance.
Caesarian and embryo transfer techniques are based on the concept of aseptically removing the pup or embryo from the relatively sterile environment of the female reproductive tract and transferring it to an MHV free foster mother or recipient in a MHV free environment. Although it has been shown that MHV can traverse the placental barrier (10), this is not thought to happen in naturally occurring infections. Both of these techniques involve the sacrifice of the donor mother. In our particular case this was not acceptable. Only a limited number of the backcross mice were available. Of these, the females were of unknown C6 status. A number of males had been identified using the C7 test as probably being C6QO heterozygotes. If caesarian rederivation or embryo transfer were to be used, at best only one litter would be available from each female, and at worst the litter could be lost. If the female was indeed C6 deficient, it is possible that it could have been mated to a normal male, or by chance produce no C6 deficient offspring in its litter. Thus it would have been wasted. With limited numbers it was decided that this posed too great a risk of the colony as a whole failing. It could have been possible to increase the size of available populations, but the time delay was considered too great.
Cross fostering and use of microisolators was considered as a viable alternative. This consists of isolating seropositive breeding pairs in microisolator caging and removing neonates to sero-negative foster mother also housed in microisolator cages. The use of microisolator caging prevents cage to cage transfer of virus particles. It seems that the design and ventilation of these cages may affect the degree of protection provided (11). Servicing of these cages requires a Class II safety cabinet. It is probable that the cross fostering itself is not actually necessary, and the use of microisolators on their own would have resulted in sero-negative young.
The individual housing of pairs means that a failure to eliminate MHV in one breeding pair would not affect any other pairs. Thus

one could risk a rest phase of only three weeks i. e. the gestation period, for the MHV virus particles to become non-infective. This system was not used due to financial constraints on equipment purchase.

Temporary cessation of breeding works on the principle that MHV is a short self-limiting infection in immunocompetent mice (12), with all animals seroconverting within a given time, and the virus particles becoming non-infective before any new susceptible pups are born. In one successful report (9), a gap of 19 weeks was used between killing of all litters and the birth of the first new litters. This results in a gap of 22 weeks with no production of young. This was considered too long a period. A second report (13) had reduced this to only 9 weeks, and was also successful in eliminating MHV. This was a more acceptable time course providing it was successful at the first attempt. The final technique used was thought to improve the chances of success while minimising disruption if it failed.

It was decided to split the colony into two. One outside the isolator would continue breeding until a MHV free colony was established. The other would be placed in the isolator as described earlier. This would result in a delay in the work of only three weeks before the isolator backcross animals were mated up. It would also mean that if this failed, a larger colony of animals would be available outside the isolator to attempt a second time possibly by a different technique.

The technique described in this paper used seropositive animals. It is presumed that seropositive animals are not excreting infective viral particles. This meant that once the animals were placed in a positive pressure isolator, the number of infective particles would decay over a period of time. The use of the Virkon bath reduced the initial MHV burden carried into the isolator. This viricidal agent was used for its' proven action and low toxicity. Twice weekly cleaning with Virkon also made the environment inhospitable to MHV survival.

MHV environmental survival time was put at 2 weeks (14). The further four weeks before susceptible young were produced allowed for detection using sentinel animals. The use of dirty litter transference as a method of detecting MHV has been established (15, 16). A period of 3 weeks for seroconversion was estimated (17). If any of the sentinel mice had seroconverted then an additional three weeks would have been added to the rest period before any litters were allowed to survive. This would have allowed a second group of sentinel animals to be used.

Although this attempt at eliminating MHV was successful, it could possibly be simplified and shortened. If it is assumed that seroconversion is 100 % by say 8 weeks of age (although this was not so in our case), then no pre-testing would be required. If viral survival is placed at 2 weeks, then pairs could be set up in the isolator immediately. This would of course increase the risk of failure, especially if large numbers of animals were transferred together (an increased risk that one is shedding actively). The use of microisolators would of course get round this, as only the cage of the shedding animal would be affected. Alternatively, one could assume that at least one animal will be shedding and at least one animal will still be susceptible. In that situation one could encourage seroconversion by grouping and litter transference.

Seroconversion is suggested to take 3 weeks, and decay of virus a further 2 weeks. By leaving the setting of pairs for two weeks, no new susceptible young stock would be introduced for the required five weeks. For any of the preceding methods not using pre-testing or sentinels to be recommended, one would have to be confident that the time course for infection, seroconversion and virus decay was appropriate for the particular mouse strain/virus strain/management system combination. The more elaborate method described in this paper although slower and more expensive, reduces the unknown and increases the certainty of success.

Summary
A colony of Peruvian mice backcrossed onto C3H/He mice was found by serological testing to be infected with MHV.
10 male and 10 female mice of the most recent backcross generation were selected and tested for antibodies to MHV. Nineteen animals were serologically positive. These were passed into a positive pressure isolator via a Virkson bath. MF1 sentinel mice in a second isolator unit were exposed to dirty litter from the backcross from day 13 onwards. Blood samples were taken from the sentinels on days 27, 34, 48 and 76. The backcross mice produced their first litter on day 43. No seroconversion occurred in the sentinel mice, or in offspring of the backcross. Animals from the original colony tested at the same age and time as the backcross offspring had seroconverted to MHV.

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