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Mice Infected with Mouse Hepatitis Virus Shed Diagnostically Detectable Amounts of IgA in Feces

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Introduction

Mouse hepatitis virus (MHV) is the most common infection in laboratory mice (Committee on Infectious Disease of Mice and Rats, 1991). It affects research in several ways, e.g. by immunosuppression and the contamination of biological research products (*Hansen,* 1994). Therefore, monitoring for MHV is mandatory in most health monitoring programmes (*Kraft et al,* 1994).

As invasive procedures in the experimental animals are undesirable, it is normal practice in health monitoring in experimental mouse units to use sentinels, i.e. animals placed on contaminated bedding inside the unit in order to catch infections. MHV easily infects sentinels (*Homberger & Thomann,* 1994), which is routinely shown by serologic testing on sera from these mice (*Hansen & Jensen,* 1995).

If mice in experiments are infected with MHV it is important to get this information as soon as possible in order to reduce both the amount of wasted work and the risk of other units becoming infected. Weekly monitoring for MHV therefore would be preferable. However, it may not be possible to place so many sentinels in a unit, that a statistically valid number of animals can be tested weekly, or it may be undesirable to bleed the same experimental or sentinel animals every week for testing purposes.

It has previously been shown that MHV-specific IgA may be found by ELISA in the stomach contents of mice (*Homberger,* 1992). As enterotropic strains of MHV may infect the entire gastrointestinal tract (*Barthold,* 1985), the epithelial plasma cells may produce significant levels of IgA. The aim of this work has been to investigate whether MHV-specific IgA may be found in the feces of infected mice. In the long run this may be used in health monitoring in experimental units.

Materials & methods

Sampling

Twenty fecal pellets were randomly sampled from 2 breeding units (A and B), as well as from four experimental units (C, D, E & F). Unit D was sampled twice. All fecal pellets were tested for MHV-specific IgA by ELISA as described below. To determine if the units were infected with MHV, sentinels (male, barrier bred Pan:NMRI mice (*Dept. Exp. Med., Panum Institute, Copenhagen,* Denmark)) were placed for 10 weeks in each unit, whereafter they were bled and euthanized. Their bedding (*Hahnflock, Bredenbek,* Germany) was changed once per week mixing the clean bedding with approximately 20 % dirty bedding from the other cages in the unit. The breeding colony delivering the sentinels (unit B) was currently health monitored according to the FELASA guidelines (*Kraft et al,* 1994). No evidence of MHV infection was present. All sera were tested for MHV by monitoring total MHV-specific immunoglobulin by ELISA as described below. In the breeding unit B mice were sampled from the breeding colonies as prescribed by FELASA (*Kraft et al,* 1994) instead of using sentinels. In unit A, in which health monitoring had shown the presence of MHV, the 15 sentinels placed in this unit were screened weekly for MHV during a period of 10 weeks. The mice were housed in five type 3 Macrolon cages (*Scanbur Ltd,* Køge, Denmark) with 3 mice in each. At introduction and weekly for the next 10 weeks each mouse was anaesthetized by a mixture of fentanyl/fluanison (*Hypnorm, Janssen Pharmaceutica,* Beerse, Belgium) and midazolam (*Dormicum, Roche,* Basel, Switzerland). Hypnorm and Dormicum was diluted 1:1 with sterile water and the dilutions mixed 1:1. Of this dilution 0,06 ml/10 g bodyweight was used for anaesthesia. Blood was sampled by periorbital puncture.

From each cage three fecal pellets were sampled weekly for monitoring MHV-specific IgA by ELISA as described below. The mice were only bled if blood could be easily obtained, and mice showing any signs of weaknesses during the observation period were euthanized.

ELISA for IgA, IgG and total MHV-specific immunoglobulin

For IgA-detection in feces one fecal pellet was placed in 1 ml phosphate-buffered saline (PBS) with 0.1 % Tween 20 and left at room temperature. After 1 hour the pellet and PBS were mixed into a homogenous suspension, which was filtered through a 0.45 µm filter (*Scleicher & Schuell,* Dassel, Germany). MHV-coated microtiter-plates for enzyme-linked immunosorbent assay (ELISA) were purchased from Charles River Wiga (*Sulzfeld,* Germany). Each ELISA-plate had 48 virus-coated wells and 48 wells coated with the cell culture, only. The plates were incubated for 2 hours at room temperature with a mixture of 50 µl of the filtrate diluted with 150 µl PBS-Tween 20 in both a well with and a well without virus. This was followed by washing 6 times with 0.1 % PBS-Tween 20 with extra NaCl (15g/l). Hereafter the plates were incubated with 200 µl horse radish peroxidase conjugated goat anti-mouse IgA diluted 1:2000 (*Organon Teknika Cappel,* Turnhout, Belgium) for 2 hours at room temperature followed by washing as above. O-phenyldiamin (OPD) (*Dako Ltd.,* Glostrup, Denmark) was used as substrate. It was inactivated by 2N sulphuric acid (*Struers KEBO Lab,* Albertslund, Denmark). Optical densities (OD) were read through a 492 nm filter and corrected by reading through a 600 nm reference filter. Over all OD-values were calculated as the difference between the OD-value of the virus-coated and the non-virus-coated well.

IgG- and total MHV-specific immunoglobulin were detected in serum diluted 1:100 in PBS by ELISA as described for IgA, in which rabbit anti-mouse IgA was replaced with goat anti-mouse IgG (*Organon Teknika Cappel,* Turnhout, Belgium), or rabbit anti-mouse immunoglobulin (*Dako Ltd.,* DK-2600 Glostrup).

A cut-off value for IgA ELISA was based upon the results achieved from all negative units, from which fecal pellets were randomly sampled. The cut-off value was set to equal the mean plus six times the standard deviation. For IgG ELISA the same formula was applied using the results achieved from the mice at introduction into unit A (week 0). Total MHV-specific immunoglobulin ELISA results were counted as positive if the difference between the OD of the coated and the non-coated well was more than 0.2, which is normal practice in our routine health monitoring.

OD-values for IgA monitoring in infected units versus non-infected units were compared by the use of analysis of variance (ANOVA).

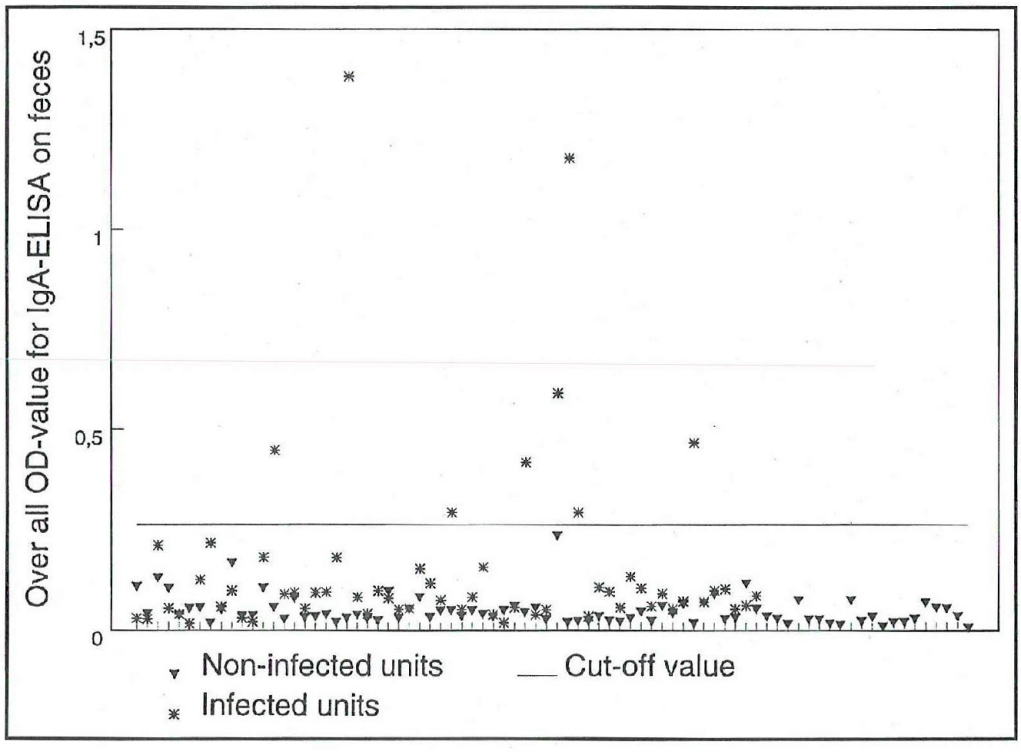
Results

Results for IgA testing in the randomly sampled feces are given in Table 1 along with the MHV-

Table 1. Results of testing 140 fecal samples for IgA antibodies against mouse hepatitis virus in ELISA compared with results from testing serum from sentinels placed in the same units for antibodies against MHV in ELISA.

Unit	MHV-specific IgA in feces	MHV-specific immunoglobulin in sera from sentinels
	Positive/tested	**Positive/tested**
A	**3/20**	**13/13**
B	0/20	0/6
C	0/20	0/6
D 1st sampling	**1/20**	**6/6**
D 2nd sampling	**4/20**	**6/6**
E	0/20	0/6
F	0/20	0/6

Figure 1. Results of ELISA for IgA against mouse hepatitis virus in randomly sampled fecal pellets. 80 pellets were from four non-infected units, while 60 pellets were from 3 infected units. The x-axis represents each of all these 140 different results. Individuals from non-infected units are marked with a triangle, while individuals form infected units are marked with an asterisk. The y-axis illustrates their over all OD-value, i.e. the OD-value of the virus-coated well minus the OD-value of the control well. Based on this figure a cut-off value was calculated as the mean plus six times the standard deviation of the results from the non-infected units, i.e. 0.263. The difference between the non-infected and the infected units is highly significant by analysis of variance ($p < 0.001$).



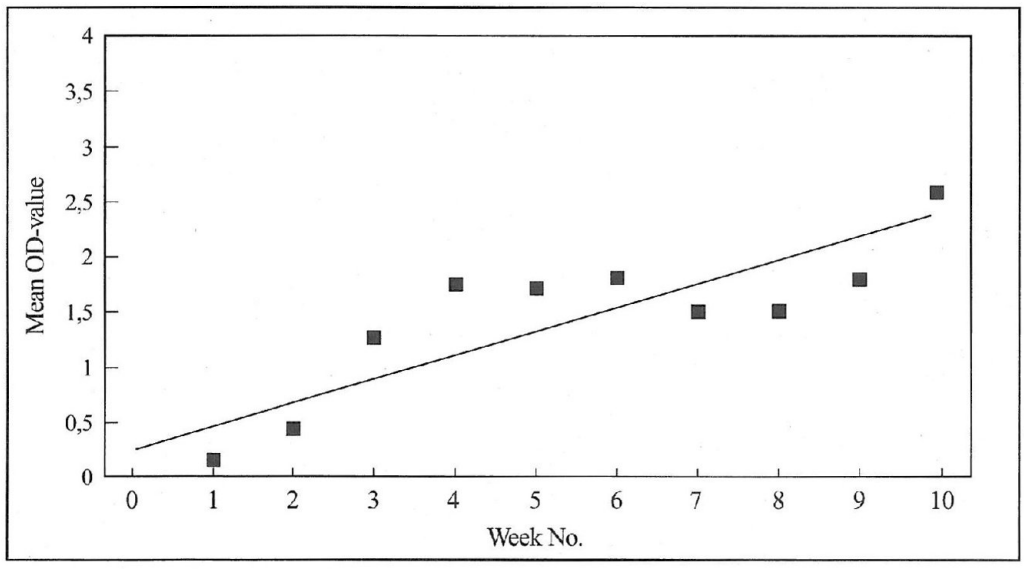
tests on sera from the sentinels. Three of the units were found to be infected with MHV. The OD-values from all IgA tests on the randomly sampled feces are given in Figure 1. IgA was low in fecal pellets from non-infected units, and significantly higher in the samples from the infected units (ANOVA, $p < 0.001$), although only eight had IgA levels above the cut-off value.

Results of ELISA for IgG in serum and IgA in feces from the current screening of sentinel mice in unit A are given in Table 2. The first IgA titer above the cut-off value was observed in week 3. The prevalence was at its maximum in week 4. Hereafter it declined and disappeared in week 10. Already in week 1 after introduction some mice had a detectable rise in IgG against MHV (Figure 2), and in week 3 the first IgG titer above the cut-off value was observed . In week 4 all mice tested had IgG titers above the cut-off value and so it remained for the rest of the observation period.

Table 2. Results (Number positive/Number tested) of ELISA for IgG in serum and IgA in feces in mice placed on contaminated bedding in a breeding unit infected with mouse hepatitis virus. Cut-off value for IgA was calculated according to Table 1, while cut-off value for IgG was calculated by the use of the mean and the standard deviation of the results achieved from the mice at introduction (Week 0), i.e. cut-off level = $\text{mean}_{\text{week 0}} + 6 * \text{s.d.}_{\text{week 0}} = 0.695$.

Week	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
IgA in feces											
absolute	0/12	0/12	0/15	**1/15**	**8/15**	**3/15**	**3/15**	**5/15**	**4/15**	**1/15**	0/15
%	0.0	0.0	0.0	**6.7**	**53.3**	**20.0**	**20.0**	**33.3**	**26.7**	**6.7**	0.0
IgG in serum											
absolute	0/15	0/13	**1/13**	**8/11**	**13/13**	**11/11**	**7/7**	**9/9**	**8/8**	**4/4**	**7/7**
%	0.0	0.0	**7.7**	**72.7**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

Figure 2. Mean IgG titers against mouse hepatitis virus (MHV) in sentinel mice kept for ten weeks in a MHV-infected unit. The curve is drawn after linear regression (OD = 0.216*week + 0.250). For numbers tested, please refer to Table 2.



Discussion

Some mice from the infected units shed clearly detectable levels of IgA in feces. This feature is usable in health monitoring. Accepting a 5 % risk of a false negative result the recommended sample size for health monitoring can be calculated from the equation

$$S = \frac{\log 0.05}{\log (1-(p*N_1))}$$

where N_1 is the nosographic sensitivity of the test

method used and p is the expected prevalence of the infection (*Hansen,* 1993). The prevalence observed in this study actually represents p^*N_1, i.e. a lower cut-off value would have resulted in a higher prevalence and thereby a lower minimal sample size, but also in a lower specificity and thereby a lower reliability of the results. The highest prevalence in the currently screened mice was observed in week 4. Four to eight weeks after introduction the prevalences ranged from 20 – 50 %, i.e. a sample of 10 fecal pellets per week should detect the infection four or five weeks after introduction of the agent. If a closed experimental unit is infected with MHV a search for IgA in a single random sample of feces may not be applicable, because all animals may be beyond the stage of fecal IgA-production. Random sampling of fecal pellets from a breeding unit already contaminated with MHV will need a much higher sample size, because these samples represent animals that have not yet seroconverted, animals which are in the most ideal period, i.e. four to eight weeks after infection, and animals which are beyond this. By such random sampling we found eight positives out of 60 samples, i.e. a sample size of at least 20 samples per sampling will be necessary. Therefore at present, fecal IgA monitoring is mostly usable for a weekly screening of mouse units. If positive results are found, the sentinels should be euthanized and blood sampled for confirmation of the results. At this time significant levels of IgG antibodies are detectable in the sentinel sera, as can be seen from Table 2. Pneumotropic MHV strains may not give rise to enteric IgA production, and therefore sentinels should still be monitored serologically for MHV every three months. IgA monitoring may also be usable for other enteric agents. However, we have also tested a huge number of fecal pellets from rats in units infected with *Kilham Rat Virus* and *Clostridium piliforme,* which did not reveal any IgA antibodies against these agents (Unpublished).

It can be concluded, that in an MHV-infected mouse colony the feces of some mice contains detectable levels of MHV-specific IgA four weeks after infection, which is usable in routine health monitoring. The advantage of monitoring IgA in feces instead of monitoring IgG in serum is, that screening can be performed with a high frequency without any invasive procedures, and without increasing the number of sentinels used. Further studies should reveal whether this is preferable to the detection of the antigen in the feces.

Summary

Fecal pellets were sampled randomly from two breeding and four experimental units for laboratory mice. 1 breeding unit and 1 experimental unit was infected with *Mouse Hepatistis Virus (MHV),* while this infection was not found in the other four units. By the use of *Enzyme-linked Immunosorbent Assay (ELISA)* it was shown that up to 20 % of the mice from the infected units had detectable levels of MHV-specific IgA in feces. By weekly screening of feces from sentinel mice from the infected breeding unit it was found, that the highest number of mice with detectable MHV-specific IgA in feces was found four weeks after infection, as more than half of the examined fecal pellets were positive during this period. Hereafter, the numbers of positives declined, until no fecal pellets containing MHV-specific IgA were found in week 10 after infection. The possibilities of using IgA monitoring in routine health monitoring are discussed.

Resumé

Fæcesprøver indsamledes tilfældigt fra to avlsenheder og fire eksperimentelle enheder med laboratoriemus. Én avlsenhed og én eksperimentel enhed var inficeret med *musehepatitis-virus (MHV),* mens de resterende enheder var fri for denne infektion. Ved brug af *enzyme-linked immunosorbent assay (ELISA)* kunne det vises, at op til 20% af musene fra de inficerede enheder udskilte påviselige mængder af MHV-specific IgA i fæces. Ved ugentlig undersøgelse af fæces fra sentinelmus i den inficerede avlsenhed observeredes det største antal mus med detekterbart MHV-specifikt IgA-niveau i fæces fire uger efter infektion, hvor over halvdelen af de undersøgte fæcesprøver var positive. Herefter faldt antallet af positive prøver. Ti uger efter infektion fandtes ingen positive for MHV-specifikt IgA i fæces. Muligheden for at anvende IgA-bestemmelse i rutinemæssig sundhedskontrol diskuteres.

References

Barthold, SW: Mouse hepatitis virus infection, intestine, mouse. In *Jones TC, U Mohr, RD Hunt* (eds.), Monographs on Pathology of Laboratory Animals. Digestive System, pp 316-312, Springer Verlag, Berlin, 1985.

Committee on Infectious Diseases of Mice and Rats: Infectious Diseases of Mice and Rats, National Academy Press, Washington D.C., p. 127, 1991.

Hansen AK: Statistical aspects of health monitoring of laboratory animal colonies, Scand. J. Lab. Anim. Sci., 20(1):11-14, 1993.

Hansen AK: Health status and the effect of microbial organisms on animal experiments, p 125-153. In *Svendsen P & J Hau* (eds.), Handbook of Laboratory Animal Science. Animal Selection and Handling in Biomedical Research, CRC Press, Boca Raton, pp 125-153, 1994.

Hansen AK & HJS Jensen: Experience from sentinel health monitoring in units containing rats and mice in experiments, Scand. J. Lab. Anim. Sci. 22(1):1-9, 1995.

Homberger FR: Maternally derived passive immunity to enterotropic mouse hepatitis virus, Arch. Virol., 122:133-141, 1992.

Homberger FR & PE Thomann: Transmission of murine viruses and mycoplasma in laboratory mouse colonies with respect to housing conditions, Lab. Anim. 28:113-120, 1994.

Kraft V, HM Blanchet, R Boot, A Deeny, AK Hansen, A Hem, H van Herck, I Kunstyr, G Melite, JR Needham, W Nicklas, A Perrot, C Rehbinder, Y Richard & G de Vroey: Recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit breeding colonies. Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Animal Health accepted by the FELASA Board of Management November 1992, Lab.Anim., 28:1-12, 1994.