Jpn. J. Infect. Dis., 52, 1999

Laboratory and Epidemiology Communications

Experiences of Microbial Contamination of Animal Colonies Maintained in the National Institute of Infectious Diseases, Japan (NIID)

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Communicated by Hiroshi Yoshikura

(Accepted January 14, 2000)

Viral and bacterial contamination of laboratory animals maintained in the NIID is reported here. *Bordetella bronchiseptica*, mouse hepatitis virus (MHV), and the hemagglutinating virus of Japan (HVJ) are the infectious agents; all of them are known to contaminate laboratory animals (1,2).

Rabbits maintained in the Murayama Branch of the NIID are regularly examined by nasal swabs for pathogens. In September 1996, *B. bronchiseptica* was detected in the nasal swab of a rabbit during a regular check of microbial infection of laboratory animals (Table 1). Necessary measures such as restriction of entry into animal laboratories were taken promptly to prevent further spread. Examination revealed that rabbits purchased from one particular breeder were infected with the bacteria. All of the118 rabbits kept in the same animal room were sacrificed. All rabbits entering the laboratory thereafter were checked for the pathogen during the next one month. With these measures, we were able to free the rabbit colony of *B. bronchiseptica*.

MHV infection occurred in a mouse breeding colony in the Toyama main campus of the NIID in October 1995. As several randomly chosen mice were found positive for MHV infection during immunological tests performed during the regular microbiological check (Table 1), entry into the room was immediately restricted. A total of 63 mice (A/WySnJ and BALB/cXid strains), each of which was taken randomly from a cage, was examined by ELISA and immunofluorescent antibody (IFA) techniques for anti-MHV antibody. For the test, we used MHV antigen, which was purchased from DENKA SEIKEN Co. Ltd., Tokyo and the affinity-purified Fab' portion of goat anti-mouse IgG (H+L-chain) (MBL, Nagoya) was used as a secondary antibody. Sixty mice (95%) were found to be MHV-infected (Fig. 1). Three months after the event, detection of the MHV genome from fresh stool specimens was attempted by reverse transcription (RT)/nested PCR (3) by using primers constructed on the sequence of the JHM strain of MHV. MHV was detected in 20 out of 37 mice tested (Fig. 2). The sequence analysis revealed homology with RI or Y strains, enteric MHV strains reported in the USA. However, our isolate was not identical to these strains. There-

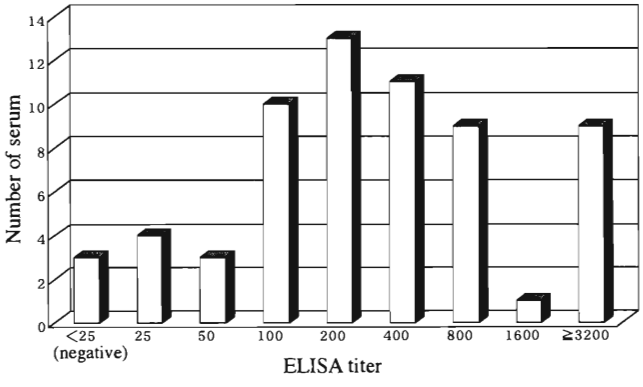
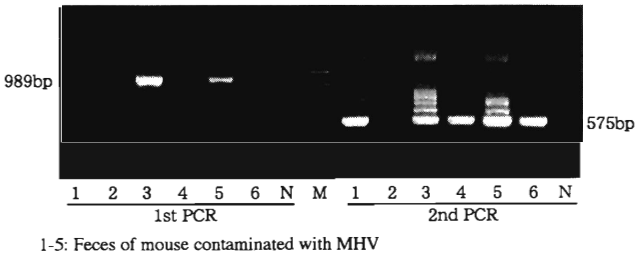


Fig. 1. MHV-specific IgG ELISA titers of sera of mice in MHV-contaminated mouse colony.



1-5: Feces of mouse contaminated with MHV
N : Feces of BALB/c mouse (negative control)
M : Marker DNA

Fig. 2. Representative results of RT/PCR assays. The six left lanes show the first PCR and the six right lanes show the second PCR. 1-5: Fecal specimens of MHV-infected mice. N: fecal specimen from normal BALB/c mice. M: Molecular weight marker.

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Table 1. Examinations for regular microbiological monitoring of mouse, rabbit and guinea pig colonies in NIID

Pathogen	Test	Mouse	Rabbit	Guinea pig
Bacteria & Mycoplasma				
Pseudomonas aeruginosa	Cultivation (NAC agar)	○	○	○
Salmonella spp.	Cultivation (DHL agar)	○	○	○
Escherichia coli O115a, c:K(B)	Cultivation (DHL agar)	○		
Pasteurella pneumotropica	Cultivation (Horse blood agar)	○		
Corynebacterium kutscheri	Cultivation (FNC agar)	○		
Tizzer's organism	Serological examination (CF)	○	○	○
Mycoplasma pulmonis	Cultivation (PPLO agar)	○		
Mycoplasma pulmonis	Serological examination (ELISA)	○		
Staphylococcus aureus	Cultivation (Mannit-salt agar)		○	
Pasteurella multocida	Cultivation (Horse blood agar)		○	
Bordetella bronchiseptica	Cultivation (DHL agar)		○	○
Streptococcus zooepidemicus	Cultivation (Horse blood agar)			○
Streptococcus pneumoniae	Cultivation (Horse blood agar)			○
Viruses				
Mouse hepatitis virus (MHV)	Serological examination (ELISA)	○		
Sendai virus (HVJ)	Serological examination (ELISA)	○		
Sendai virus (HVJ)	Serological examination (CF)		○	○
Ectromeria virus	Serological examination (ELISA)	○		
Parasites				
Giardia muris	Microscopic examination	○		
Spironucleus muris (*Hexamita muris*)	Microscopic examination	○		
Syphacia spp.	Microscopic examination	○		
Cysticercus fasciolaris	Visual examination	○		
Myobia musculi	Microscopic examination	○		
Radfordia affinis	Microscopic examination	○		
Eimeria spp.	Microscopic examination		○	
Cysticercus pisiformis	Visual examination		○	
Psoroptes cuniculi	Microscopic examination		○	
Eimeria cavise	Microscopic examination			○

Table 2. Detection of antibody in sera of mice infected with A, B or C strain of virus

Virus strain	Mouse strain	No. of positive/No. of animals examined			
		Anti-A	Anti-B	Anti-D	Anti-Sendai virus
A	BALB/c	6/6	NT[1)]	NT	6/6
B	BALB/c	0/5	5/5	NT	0/5
D	C3H	NT	NT	5/5	0/5
	BALB/c	NT	NT	5/5	0/5

[1)]Not tested

fore, our isolate was referred to as the TY strain (unpublished data). The data indicated the continued persistence of MHV in the colony.

Contamination of a mouse colony with Sendai virus occurred during an experiment on the adaptation of influenza virus to mice. The virus was serially passaged in mice by pulmonary infection. During the experiments, control mice suddenly started to die. Microbiological examination of the dead control mice revealed a rise in anti-Sendai virus antibody. As Sendai virus infection was suspected, all of the mice were sacrificed and entry into the room was restricted. The room was thoroughly sterilized. However, when the experiment was reinitiated, Sendai virus infection reappeared; among the 42 mice kept on the same rack, 37 (88%) were anti-Sendai virus positive. Contamination by Sendai virus in the inoculum influenza virus stock was suspected. In fact, one of the virus stocks was found to induce anti-Sendai virus antibody in mice after injection (Table 2). Sendai virus could be removed from the virus stock by mouse passages under the presence of anti-Sendai virus antibody (×163,840 in an ELISA test) prepared in rabbits by using UV-inactivated Sendai virus. Sendai virus infection of the mouse colony has not reoccurred.

Thus, these experiences underscore the importance of regular microbiological monitoring (Table 1) of laboratory animals and of prompt reactions to prevent the spread of pathogens.

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