Molecular detection of mouse hepatitis virus in laboratory mouse colonies

Roozbeh Fallahi, Fatemeh Abedini, Gholam Reza Shokri

a Department of Research, Breeding and Production of Laboratory Animals, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran.

b Research and Development Laboratory, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

ABSTRACT

The animal health monitoring is required to issue health certificates. The viral hepatitis virus is one of the most important infectious agents in mice breeding colonies. This research used RT-PCR to identify contaminations to mouse hepatitis virus. 18 out of 29 specimens were found to be infected, a prevalence of 62%. PCR product was purified and sequenced. Phylogenetic analysis revealed that the identified strain in this study was closely related to a strain reported from France. In the conventional system, contamination with different infectious agents is inevitable, thus it is better to replace the contaminated colonies with clean animals.

Keywords: Molecular detection, Mouse, Hepatitis virus

Abbreviations:

MHV: Mouse hepatitis virus
ELISA: Enzyme linked immunosorbent assay
RT-PCR: Reverse transcription-polymerase chain reaction
NIH: National Institutes of Health
NC: Nucleocapsid
FELASA: Federation of European Laboratory Animal Science Associations
IVC: Individually ventilated cage

www.IJVST.um.ac.ir

DOI: 10.22067/veterinary.v11i2.77631
Mouse hepatitis virus is a common infection in a large number of laboratory mouse colonies and is known to interfere with research results (1). MHV is an enveloped virus which has a 31Kb single-strand positive RNA genome. MHV belongs to the Coronaviridae family and replicates in the cytoplasm of infected cells using a viral RNA-dependent RNA polymerase which is translated from the genomic RNA (2, 3). MHV strains are classified as respiratory tropic or enterotropic groups based on tissue distribution of primary infection (2, 4), although the enterotropic infection is considered to be the most common from of infection (10). MHV is well known to be the most common virus of laboratory mice (2, 5). Natural infections with MHV remain widespread in most laboratory mouse populations despite the efforts to detect and eradicate this agent (6). Current data based on serological tests estimate that 60 to 80% of laboratory animal colonies are infected with MHV (2). Since its first description by Cheever in the late 1940’s, MHV has been shown to alter the results of in vivo experiments using other infectious and non-infectious agents (2, 7). Concomitant infection with MHV has been correlated with altered responses to tumours (8) and to other viruses (2). Also, immune system modulation experiments were noted to potentiate MHV infection and disease (2, 7). MHV is able to spread rapidly in mouse colonies because of its high contagiousness (2, 9, 10). Therefore an early detection of MHV infection is very important. Current methods which are used to detect MHV infection include ELISA and immunofluorescence techniques. The diagnosis of MHV infection is mainly performed by serological assays due to the difficulties in finding histological lesions and in isolating the virus in tissue culture (2, 11). However, the seroconversion of the animal sentinels or the newly infected ones requires a waiting period before a serologic assay can be used. The direct detection of viral nucleic acid using molecular biology methods in clinical or necropsy specimens would be a quick and powerful means to detect an outbreak or a sub-clinical condition affecting the animals (2, 11-14). RT-PCR has been effective in the detection of MHV in tissues and feces of infected mice (10, 12, 22). The aim of this study was to evaluate mouse hepatitis virus in NIH mice colonies in one laboratory animal facility in Iran using RT-PCR method.

According to the FELASA instruction, taking into account a 10% prevalence of contamination and 95% confidence, 29 samples was needed. In this study, NIH breeding mice from both sexes in the breeding room were randomly selected and monitored for mouse hepatitis virus according to the ethical protocols. Samples were collected from the intestine (colon) containing feces and prepared by standard methods. Then, RT-PCR was performed (10, 11, 12, 22). The sequence of NC gene (F: 5’- CAGCAGTTTTGGAAAGAGAG-3’, R:5’- TGGCCTTGGCAACGCTTA-3’)(2) available in the Genbank (Accession number, EMBL: AB351247.1) were cloned in pUC57 vector (CinnaGen, Tehran, Iran). The pUC57-NC plasmid was used as positive control. Plasmid extraction was performed by the GF-1 kit (Vivantis, Malaysia) in accordance with the protocol. RNA was extracted from intestinal tissue samples using Trizol (25). Contaminating genomic DNA was removed by Dnase I (Fermentas, ... treatment) (25). The conversion of RNA to cDNA was carried out using the Viva 2 steps RT-PCR kit (Vivantis, Malaysia) (25). The reaction was carried out with a final volume of 25 μl according to the protocol (26). The PCR reaction included: initial denaturation at 94 °C for 5 minutes, 30 cycles of denaturation at 94 °C for 1 minute, annealing at 52 °C for 1 minute, and extension at 72 °C for 1 minute, and a final extension at 72 °C for 10 minutes (26).

In 18 colon samples containing feces, the infection to MHV was positively detected. Therefore, the prevalence of this infection was calculated to be 62% (Figure 1).

For phylogenetic study, the positive sample was tested 3 times. Therefore, a PCR product was sequenced by BIONEER (South Korea). The alignment study was conducted through the EMBL-EBI and Klign (2.0) program and the sequence acquired from Sanger sequencing was compared with the sequences of the four other species obtained from the NCBI GenBank. In the phylogeny tree, the strain KX774640: 0.04601 belongs to this study and other species are X63538: 0.03507, L37760: 0.02799, L37759: 0.01186 and L37758: 0.02207, respectively. The most closely related strain in this study has been shown to be X63538: 0.03507 in France. The degree of affinity is found in the phylogeny tree (Figure 2).

Based on the recommendation of the FELASA, animal health monitoring is required to issue health certificates that are required for quality systems and quality control of production and research institutes (11, 15, 16). Many infectious agents in laboratory animals cause infections in humans and they are zoornoses (17, 18). Recommendations should be based on individual and local needs, considerations of research work, factors that are prevalent regionally, and national goals that are relevant in each country (11, 15, 16). The transmission of infectious agents and the presence of allergenic agents in open-cage systems are more prevalent than closed systems. Thus, it is much more important to carry out health monitoring programs in conventional open-cage systems. (11, 15, 16). In Iranian laboratory animal breeding centers, despite the advances made in design and breeding methods,
some infections, especially parasitic, bacterial and viral infections, are still present. Although clinical symptoms may not be seen in contamination with infectious agents, it can negatively affect the quality of the vaccine and biological products tested in these animals (11, 15).

In this study, the prevalence of this infection was 62%. Despite the advances made in the design and method of breeding centers especially in this center, the prevalence of this viral infection is still high. Although there are no clinical signs of contamination with these virus, it can negatively affect the results of the research and quality control tests. In the conventional system, contamination with different microbial agents is inevitable, but it is better to replace the contaminated colonies with clean animals. There are many reports on the health surveillance of viral infectious agents in foreign countries. The first description of the mouse hepatitis virus was provided by Cheever et al. (1949) (16). Parker (1979) identified 60-80% infection rates of mouse hepatitis virus in laboratory animals (17). Kagiyama et al. (1986) introduced the mouse hepatitis virus as one of the common viruses in laboratory mice (5). Homberger et al. (1991) and Yamada et al. (1993) introduced the RT-PCR as a suitable method for detecting the mouse hepatitis virus (18, 19). Yamada et al. (1993) announced that the virus could rapidly spread to the laboratory colonies due to easy transfer through contaminated materials (19). Adami et al. (1995) and Barthold and Smith (1990) published reports of viral hepatitis infection in mice and rats in both animal and wildlife animal breeding centers (3, 7). Jacoby and Lindsey (1997) reported the hepatitis mouse virus in 60% of the conventional breeding centers and 10% of the eligible systems of the barriers (20). Cecilio et al. (2000) detected mouse hepatitis virus by Nested PCR in liver tissue samples of laboratory mice (2). Matthaei et al. (1998) used the polymerase chain reaction to diagnose a natural outbreak of mouse hepatitis virus infection in nude mice (21). Oyanagi et al. (2004) detected the MHV-RNAs in mouse intestines and in filter dust in mouse room ventilation duct by a modified RT-nested PCR (22). Wang et al. (1999) diagnosed the mouse hepatitis virus contamination in nude mouse population by using RT-PCR (23). Nowadays, the large production and breeding centers are tested for the diagnosis of mouse hepatitis virus by PCR every six weeks (24). In Iran, there have been no investigations into this virus. Fallahi and Mansouri (2017) reported the health monitoring of NIH laboratory mice to Clostridium piliforme (24). The use of filter cages in the IVC breeding system prevents the transmission of airborne contamination. Although infection with the virus is unusual in humans, full compliance with health rules is required for staff working with rodents.

Acknowledgment
The author would like to thank the staff of Department of Research, Breeding and Production of Laboratory Animals, Razi Vaccine and Serum Research Institute for their kind cooperation.

Author Contributions
RF prepared the experimental design, wrote and revised the article, and managed the research. FA performed the experiments.

Figure 1
Agarose gel electrophoresis of RT-PCR products from Mouse Hepatitis Virus, NC gene. [M-100: PCRBIO Ladder IV DNA Marker- 100 bp (Arian Gene Gostar), Con-: Negative Control, Con+: Positive Control, NS: Negative Sample, PS: Positive Sample]

Figure 2
The phylogenetic tree of the strain detected in this research (KX774640:0.04601) As shown in the picture, the most closely related specie is X63538: 0.03507 from France.
Conflict of Interest

The authors declare that they have no conflict of interest.

References


تشخیص مولکولی ویروس هپاتیت موشی در کلنی موش‌های آزمایشگاهی

روزبه فلاحی، فاطمه عابدینی، غلامرضا شکری

چکیده
پایش بهداشتی حیوانات، جهت صدور گواهی سلامت آنها الزامی است. ویروس هپاتیت موشی از مهم‌ترین عوامل عفونی در کلنی محصولات، بخش تحقیق، تولید و پرورش حیوانات آزمایشگاهی، موسسه تحقیقات واکسن و سرم‌سازی رازی، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران

پاپیرون کد پالسیژنی

ویروس‌های آزمایشگاهی تولید می‌شوند، برای تشخیص موش‌های آزمایشگاهی در بخش تحقیق، تولید و پرورش حیوانات آزمایشگاهی، موسسه تحقیقات واکسن و سرم‌سازی رازی، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران به روش‌های جدیدی می‌پردازند. در این مقاله، از روش PCR و تکنیک RT-PCR برای تشخیص ویروس‌های آزمایشگاهی استفاده می‌شود. در این روش، 62 نمونه از موش‌ها مبتلا به ویروس هپاتیت موشی در کلنی محصولات، بخش تحقیق، تولید و پرورش حیوانات آزمایشگاهی، موسسه تحقیقات واکسن و سرم‌سازی رازی، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران پایش گردید. در درخت فیلوژنی، سویه متعلق به این تحقیق بیشترین قرابت را با سویه ای از کشور فرانسه نشان داد. در سیستم‌های پرورش متعارف، آلودگی با عوامل مختلف میکروبی اجتناب ناپذیر است. پس از آنکه بهترین انتخاب این است که با کلنی‌های آلوده‌ای سایر بهترین انتخاب با کلنی‌های آلوده‌ای سایر