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Disease Surveillance in Mouse Colony Using Indirect Sentinel Animal Program at Laboratory Animal Center at Thammasat University

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ABSTRACT

Surveillance of communicable diseases among laboratory animals is an important component of veterinary care within any animal research facility. This study aimed to monitor the health status of research animals at the Laboratory Animal Center, Thammasat University, using the Sentinel Animal Program. Five female, specific pathogen free, BALB/cMlac mice were designated as sentinel animals. After a 3-day quarantine, the sentinel mice were housed in a cage which was placed in the same laboratory-animal room with 50 other experimental mice. An indirect sentinel test was performed twice weekly for six weeks by transferring soiled bedding from different cages of the experimental mice into the cage of the sentinel mice. After six weeks, the sentinel mice were euthanized. Their blood samples and intestinal contents were tested to detect any evidence of infections by certain pathogens. Their organ tissues were also reviewed under microscope. By using ELISA, cultivation and examination under microscope, no evidence of infections was detected among the sentinel mice. Their organ tissue histology appeared normal. The sentinel mice did not receive any infectious diseases, if present, from the experimental mice. This reflects the generally good health status of other experimental mice simultaneously housed in the same facility. However, it should be noted that certain pathogens cannot transmit via soiled bedding and, hence, may not be able to be detected under the Sentinel Animal Program using the indirect contact method.

Keywords: Animal health monitor; BALB/cMlac mice; Sentinel animal; Specific pathogen free

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1. Introduction

The health status of experimental animals is an important component in any biomedical research using animals. Even minor alteration of animal health can have a direct impact on the overall reliability of research data. Diseases among experimental animals are classified into three categories: multifactorial diseases, epidemic diseases, and transmissible infectious diseases [1]. Whereas the former two categories are associated predominately with large-scale settings such as livestock farms, transmissible infectious diseases can frequently appear in any susceptible animal population, including animals housed in research units [1].

Any health problem of laboratory animals, whether fatal or not, can directly interfere with research data. The reliability and integrity of any animal research can be maintained by early detection of any abnormality among laboratory animals. In rodents such as mice, rats, hamsters and guinea pigs, infections by several pathogens often do not show obvious clinical signs. Moreover, some animals become a reservoir of the pathogens. These infections, if left undetected, can affect the physiological status of the animals and, consequently, the whole research outcome [2]. Some pathogens can even cause zoonosis in humans. A comprehensive program for monitoring transmissible infectious diseases among experimental animals is thus a vital part of the veterinary care within any research facility.

Founded in 2010, the Laboratory Animal Center at Thammasat University (LAC-TU) has the mission of providing husbandry and taking care of laboratory animals for scientific purposes. All processes comply with The Guide Care and Use of Laboratory Animals [3]. Recently, LAC-TU has housed and taken care of a colony of mice over a period of six months. Besides routine health monitoring, it was recommended that disease surveillance be applied in order to detect the presence of any disease or infection which may occur among these mice [4]. The surveillance is part of the health monitoring program according to the Guide of Federation of European Laboratory Animal Science Association (FELASA) and it aims to maintain the quality of health status of laboratory animals during the entire experimental course [4].

The Sentinel Animal Program is the disease surveillance program designed to represent the health status of experimental animals. The sentinel animal is an animal of the same species as the experimental animals, which is placed in the same environmental setting [5]. The objective of this study was to conduct a comprehensive health monitoring program, and the Sentinel Animal Program was chosen as the study method.

2. Materials and Methods

2.1 Ethical approval

The present study was approved by the Institutional Animal Care and Use Committee, Thammasat University (IACUC-TU). The approval number is 003/2559. The Laboratory Animal Center at Thammasat University has had AAALAC accreditation since June 2016.

2.2 Animals and sample size calculation

The sample size of the sentinel animals was derived from the following equation [6]:

$$\text{Sample size} = \log 0.05 / \log N$$

where N = percentage of non-infected animals; 0.05 = 95% confidence level.

By calculating the confidence level at 95% and prevalence rate at 30%, the sample size was 10 sentinel animals per 100 experimental animals. In our institute at the time of the study, there were 50 experimental mice housed in the animal-husbandry room. Five animals were thus

recruited to be the sentinel animals for this study. Specific-pathogen-free, inbred strain BALB/cMlac, female mice (*Mus musculus*) at the age of 8-9 weeks and weight of 20-25 grams were chosen. All animals were purchased from the National Laboratory Animal Center (NLAC), Mahidol University, and were transferred in a ventilation-controlled truck. The animals were acclimatized in an environmentally controlled quarantine room for 3 days before being brought to the same animal room housing the 50 experimental mice. Animals showing any abnormal signs during the quarantine period were removed.

In the animal room, the five sentinel mice were housed in a 20.7 x 36.5 x 14 cm cage, made from polysulfone with corncob bedding. The sentinel animal cage was located at the lower right of the rack, separated from the usual experiment cages. The sentinel animals were housed in a cage with the safe harbor mouse retreat as an environmental enrichment and were allowed to feed and water *ad libitum*. The bedding was changed twice weekly. To represent the microbiological status of the experimental mice, the sentinel mice were housed in the same room for six weeks.

2.3 Indirect sentinel test

An indirect sentinel test was performed according to the American Association for Laboratory Animal Science (2007) [7]. In brief, a spoonful of soiled bedding from the cages of the experimental mice was transferred into the cage of the sentinel mice twice weekly (on Tuesday and Friday) (Fig. 1). We used soiled bedding from a different cage every time we performed the transferring and alternatively rotated the cages for soiled bedding until the end of the study period.

2.4 Microbiology and Parasitology studies

At the end of the study period, the sentinel animals were anesthetized by 3.5% Isoflurane with 0.9 liters of oxygen within an induction chamber and then maintained under anesthesia with 1.5% Isoflurane with 0.4-0.8 liters of oxygen under mask. Their blood samples were collected and sent to NLAC, Mahidol University for detection of pathogenic viruses including *Sendai virus* (*SeV*) and *Mouse hepatitis virus* (*MHV*) and bacteria including *Mycoplasma pulmonis* and *Clostridium piliforme* using ELISA. All sentinel animals were then euthanized with inhaled carbon dioxide following the method suggested by American Veterinary Medical Association Guidelines for the Euthanasia of Animals [8]. Death was confirmed by the absence of heartbeat, movement of the chest and reflexes as well as the presence of pale mucous membrane. Ectoparasites and parasitic eggs and larvae were detected around perianal area using hair pluck examination (tape test) [9] and were examined under microscope. Hair samples were collected and cultured on Dermatophyte selective agar at 28-30^{o}C for up to 3 weeks. To detect respiratory pathogens, a laryngotracheal swab was performed and samples were cultured on PPLO (pleuropneumonia-like organisms) selective agar at 37^{o}C for 7 days as well as on blood agar and DHL (Deoxycholate Hydrogen sulfide Lactose) agar at 37^{o}C for 1-2 days. To detect gastrointestinal pathogens, tissues at caecum were collected and cultivated on Baird-Parker agar, DHL agar and Cetrimide agar at 37^{o}C for 1-2 days. Duodenum and caecum contents were also examined under microscope.

2.5 Necropsy and Histopathological studies

All animals were examined and thoroughly searched for any superficial lymphadenopathy and hemorrhagic lesion. The euthanized sentinel animals had their skin surface disinfected with 70% alcohol before dissection. Superficial lymphadenopathy and hemorrhagic spots were recorded. Dissections of abdomen and peritoneal areas, as well as the thoracic cavity, were

performed. Organ size and appearance were recorded.

Fig. 1. Indirect sentinel test by transferring a spoonful of soiled bedding from the cage of the experimental mice (a) into the cage of the sentinel mice (b).

Tissue samples were collected and stored in 10% neutral buffered formalin. The necropsy samples were sent to NLAC, Mahidol University for histopathological studies.

3. Results and Discussion

3.1 Microbiological and Parasitological status

The present study collected blood samples and organs including heart, lung, kidney, liver, spleen, intestine and caecum of the sentinel animals and tested for the presence of pathogens commonly occurring among laboratory animals. By using ELISA to detect the presentation of pathogenic viruses and bacteria, no serum sample was found to be positive for *Sendai virus*, *Mouse hepatitis virus*, *M. pulmonis* or *C. piliforme*. All cultivation samples including laryngotracheal swabs, caecum tissues, duodenum and caecum contents, and hair samples showed no growth in all culture media. No ectoparasites or parasitic eggs or larvae were found in the microscopic examination of the perianal samples from hair pluck examination (tape test) and from the smears of duodenum and caecum contents. Table 1 shows results of the selected specific pathogens that were tested and/or sought in the present study incorporated with their pathogenic potential category to which they belong.

The aerosol transmissible pathogens tested in this study included *Sendai virus* (*SeV*) (old name is *Murine Parainfluenza virus* type I), *Mycoplasma pulmonis*, cilia-associated respiratory (CAR) bacillus. *SeV* is a highly transmissible pathogen that causes respiratory diseases among immunocompetent laboratory animals, especially mice. It can cause acute sudden death in pre- and post-weaning mice. Rats, hamsters, and guinea pigs are also susceptible to the *SeV* infections [10]. *M. pulmonis* is a pathogen responsible for murine respiratory mycoplasmosis which can infect immunodeficient and/or stressed rats and mice [10]. CAR bacillus is a highly contagious gram-negative bacteria that can cause respiratory infections in rats, mice and rabbits. Other bacteria occasionally associated with respiratory tract infections including *Pasteurella pneumotropica* and *Corynebacterium kutcheri* were also included in this study. These pathogens are responsible for conditions manifesting with pneumonia, difficult breathing, teeth chattering, weight loss and conjunctivitis in mice [11].

This study also conducted the detection of common pathogens transmissible via fecal-oral route including *Citrobacter rodentium, Clostridium piliforme* and *Salmonella* spp. *C. rodentium* is responsible for transmissible murine colonic hyperplasia (TMCH) which can present with diarrhea, retarded growth, ruffled hair, soft feces and rectal prolapse. TMHC often occurs in weaned mice, leading to a high mortality. Contaminated food, water, or bedding are possible source of the infection [10]. *Clostridium piliforme*, a spore-forming bacteria, is the cause of Tyzzer's disease and can infect laboratory animals such as mice, rats and rabbits. Tyzzer's disease occurs more frequently in immunosuppressed animals as well as animals housed in unfavorable and poor conditions. It is transmitted by eating feces of infected animals or by receiving the spores. *Salmonella* spp. are also found among animals in unfavorable and poor housing conditions. Importantly, *Salmonella* spp. can spread from animals to human [10].

Common pathogens causing diseases in both animals and humans, including *Pseudomonas aeruginosa* and *Staphylococcus aureus,* were also tested in this study. *P. aeruginosa* can cause a lethal infection. It is frequently found in association with untreated drinking water, and it may colonize the oropharynx, nasopharynx and gastrointestinal tract of animals. Infected animals may succumb from rapid septicemia, which has clinical manifestations ranging from listlessness, conjunctivitis, serosanguinous nasal discharge, edema of the head, anorexia, and death [10]. *S. aureus* commonly infects the skin and mucus membranes of animals. This pathogen is most frequently associated with abscesses, conjunctivitis and superficial pyoderma particularly around the head and face of animals [10].

A detection test of *Mouse Hepatitis Virus* (*MHV*) was also conducted. *MHV* is highly contagious and can cause necrotizing hepatitis. It can spread by respiratory transmission, fecal-oral transmission, or direct contact. Contaminated feces and nasopharyngeal exudates as well as fomites can serve as a source of infection [11].

3.2 Necropsy and Histopathology findings

At the end of the Sentinel program, gross necropsy examination was thoroughly performed in all mice. No superficial lymphadenopathy or hemorrhagic lesions were found.

Histopathological study of renal tissues (Fig. 2a-c) revealed a few atrophic glomeruli and proximal tubules in the renal cortex. Corticomedullary and medullary tubules were unchanged whereas a few papillary tubules were slightly dilated without any type of cast. These findings may occur without any apparent cause. Slight vasodilation and congestion within the vessel in the renal pelvis mucosa were observed. Adipose tissue adhesive at the renal capsule was found. Overall, renal histology was otherwise intact with some minor structural anomalies which could be regarded as normal histologic variation.

Histopathological study of the heart revealed clear cytoplasmic vacuolar changes in cardiomyocytes in one small area in the ventricle (Fig. 2d) while the rest of the cardiac tissue was intact. The vacuolation may be associated with an accumulation of lipid, but this finding appeared in the small area and did not result in any detectable physiologic changes of tested mice. The atrium was unchanged but some parts lost tissue texture. The aorta was filled with red blood cells. Valves and endocardium had no inflammatory cell infiltration or progressive lesion to fibrosis.

Histopathological studies of the liver revealed moderate diffuse micro-vacuoles deposits in the cytoplasm of hepatocytes (Fig. 2e). This condition could result from a mild lipid accumulation. In spleen tissue, mild brownish deposits, most likely hemosiderin, were found scattered within

otherwise normal splenic parenchyma (Fig. 2f). Overall, there were merely minor non-specific histologic changes in the tissues of the sentinel mice.

In this study, we could not detect any pathogens or any pathological changes from the sentinel mice. The sentinel mice were housed for six weeks in the same condition with the experimental mice of interest. They indirectly contacted the experimental mice when a spoonful of soiled bedding from the cage of the experimental mice was transferred into the cage of the sentinel mice. This method is called "*indirect sentinel test*" and was performed twice weekly over a period of six weeks. Findings that no pathogen was detected suggest the generally good health status of the experimental mice of interest. Factors which may contribute to these findings include the quality of the primary enclosure (i.e. food, water and bedding) and the efficacy of the secondary enclosure (i.e. air ventilation system). At our facility, food, water and bedding are prepared according to Guide for the Care and Use of Laboratory Animals [3], and the air ventilation system is controlled by using HVAC (Heating Ventilation Air Condition) system.

Table 1. Results of the specific pathogens tested and/or sought with their category.

Pathogen	Sample	Detection Method	Result	Category*
Sendai virus	Serum	ELISA	not found	B
Mouse hepatitis virus	Serum	ELISA	not found	B
Clostridium piliforme	Serum	ELISA	not found	C
Mycoplasma pulmonis	Serum	ELISA	not found	C
Mycoplasma pulmonis	Laryngotracheal swab	Culture	not found	C
Corynebacterium kutscheri	Laryngotracheal swab	Culture	not found	C
Pasteurella pneumotropica	Laryngotracheal swab	Culture	not found	C
Streptococcus pneumoniae	Laryngotracheal swab	Culture	not found	C
Staphylococcus aureus	Laryngotracheal swab	Culture	not found	D
Salmonella spp.	Gastrointestinal content	Culture	not found	A
Citrobacter rodentium	Gastrointestinal content	Culture	not found	C
Pseudomonas aeruginosa	Gastrointestinal content	Culture	not found	D
Microsporum sp.	Pelage	Culture	not found	A
Thicrophyton mentagrophyte	Pelage	Culture	not found	A
Syphacia spp. larvae	Gastrointestinal content	Direct examination	not found	E
Syphacia spp. egg	Gastrointestinal content	Direct examination	not found	E
Ectoparasites	Skin	Direct examination	not found	E

Note:
*Category A = Zoonotic and human pathogens carried by mice and rats.
Category B = Fatal pathogens to mice and rats.
Category C = Potential pathogens capable of causing diseases in mice and rats and affecting their physiological function.
Category D = Opportunistic pathogens of mice and rats.
Category E = Microbes as indicators of the microbiological and hygienic status of the mice and rats.

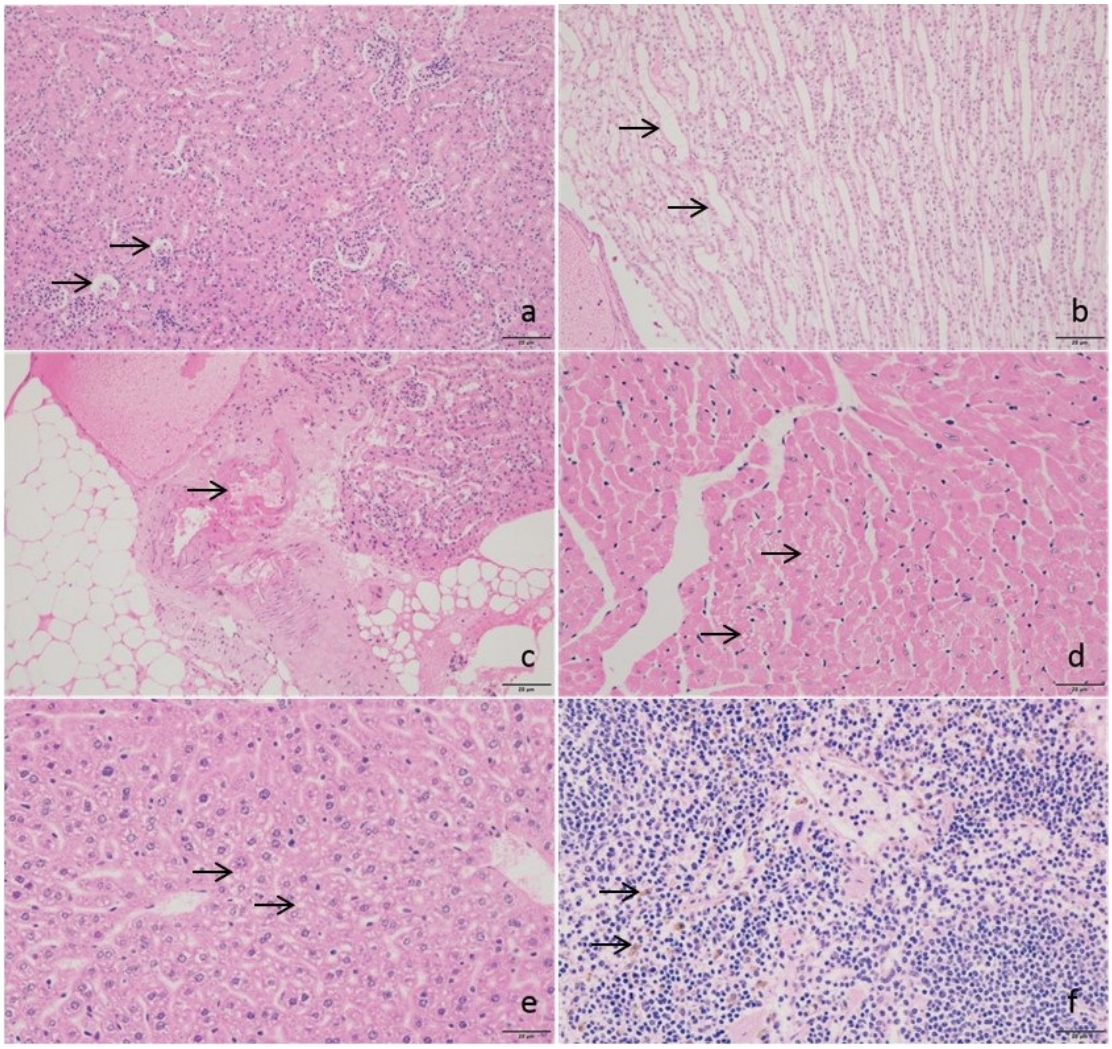

Fig. 2. Histopathology of (a) a few atrophic glomeruli among intact glomeruli, (b) slightly dilated papillary tubules, (c) vasodilation and congestion at wall of renal pelvis, (d) focal vacuolation in cardiomyocytes, (e) diffuse micro-vacuole in hepatocytes, and (f) brown pigments in splenic parenchyma.

4. Conclusion

The sentinel animals have been widely recommended as a tool to monitor the presence of diseases or infections. By using animals of the same species and strain as the animals of interest, these sentinels can indicate in a timely fashion the health status of the study populations.

This study employed the Sentinel Animal Program, according to the guidelines of the Federation of European Laboratory Animal Science Association (FELASA), to monitor the health status of the laboratory mouse colony housed at our institute. By housing five Mlac BALB/c mice as sentinel animals for six weeks and performing the indirect sentinel test, we found no detected pathogen or any abnormal histopathology in the sentinel animals, representing the microbiological status of the experimental mouse colony at that time. This study emphasizes the importance of the health monitoring system using the Sentinel Animal Program.

While the *direct sentinel animal test* may yield a more comprehensive result, it is not always feasible to conduct such a test as it needs additional animals being housed in the same cage with the experiment animals. The *indirect sentinel animal program*, on the other hand, can be conducted in addition to the ongoing research without disturbing the experiment animals. Besides its low cost and feasibility, the *indirect sentinel animal program* is an appropriate and effective surveillance strategy especially for monitoring the presence of pathogens transmitted via fecal-oral route.

The limitation of the method used in this study may affect our findings. It is important to note that the *indirect sentinel test* cannot convey some pathogens from the experimental animals to the sentinel animals [11]. For example, *SeV* is transmitted via aerosol and direct contact while CAR bacillus is transmitted through only through direct contact. Thus, the *indirect sentinel test* may not be the most efficient means for detecting these pathogens. Moreover, it is possible that the amount of the pathogens passed through the soiled bedding may be not sufficient to infect the sentinel animals. Negative findings of the *indirect sentinel test* should be carefully interpreted.

To ensure the thoroughness of the animal health monitoring, the *direct sentinel test*, which allows sentinel animals directly contact with experimental animals, may be added. Even though the current report did not conduct the *direct sentinel test*, we have performed this test occasionally on the animal colonies being housed in the facility for six months or longer. To date, three animals (two mice and a hamster) have been tested on three different occasions. No significant abnormality or pathogens were detected in mice, whereas a pear-shaped flagellate was found in the hamster, but it was most likely a commensal organism.

Recently, a new monitoring method called “Exhaust Air Dust Monitoring” has been introduced and is claimed to be superior to the soiled bedding sentinel [12]. It may become an alternative health monitoring method in the future; however, it is expensive to conduct this testing program and it can be applied to animals in an individually ventilated cage (IVC) only.

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