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DETECTION OF PARVOVIRUS IN A SENTINEL ANIMAL REPLACEMENT TRIAL IN HEALTH MONITORING USING SOILED BEDDING SWABS FROM EXPERIMENTAL ANIMALS HOUSED IN VENTILATED CABINETS

DETECÇÃO DE PARVOVIRUS EM UM ENSAIO DE SUBSTITUIÇÃO DE ANIMAIS SENTINELAS EM MONITORAMENTO DE SAÚDE USANDO SWABS DE CAMA SUJA DE ANIMAIS EXPERIMENTAIS ALOJADOS EM ARMÁRIOS VENTILADOS

DETECCIÓN DE PARVOVIRUS EN UN ENSAYO DE SUSTITUCIÓN DE ANIMALES CENTINELA EN EL CONTROL SANITARIO USANDO HISOPOS DE CAMA SUCIA DE ANIMALES DE EXPERIMENTACIÓN ALOJADOS EN GABINETES VENTILADOS

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ABSTRACT

Laboratory animals are exposed to infection by a variety of pathogens that are widely recognized as adverse factors in biomedical research. According to international regulations, these microorganisms must be absent from experimental rat and mouse colonies. Experimentation with sentinel animals exposed to contaminated bedding from experimental mice is the most common method for sanitary monitoring in animal facilities. Although environmental sampling is being explored and, in many cases, has been implemented as an alternative, sampling of exhaust air from ventilated cabinets (IVCs) is not effective in controlling all pathogens that must be excluded from animals used in trials. Among the murine viruses present in mouse colonies, the Minute Virus of Mice (MVM) stands out. This virus belongs to the Parvoviridae family and is considered highly prevalent in mouse colonies and a contaminant of some virus stocks and transplantable tumors. Like all parvoviruses, the dependence of MVM replication on functions modulated by cellular proliferation, differentiation, and transformation indicates that the virus requires dividing cells to replicate. Replication occurs in various organs such as the pancreas, small intestine, lymphoid organs, liver, and kidneys. This virus is shed and transmitted through feces and urine, where it can persist for several weeks. In this experiment, PCR tests were evaluated for the detection of MVM in swabs placed on contaminated bedding in sentinel cages in an IVC for sanitary monitoring in laboratory mouse colonies. All samples tested positive for MVM. The implementation of swabs can be used to replace the use and slaughter of sentinel animals and establish a sanitary monitoring program for prevalent pathogens in production and research vivariums

Keywords: Replacement. Refinement. Mice. Health Control.

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RESUMO

Animais de laboratório são expostos à infecção por uma variedade de patógenos amplamente reconhecidos como fatores adversos em pesquisas biomédicas. De acordo com regulamentações internacionais, esses microrganismos devem estar ausentes das colônias experimentais de ratos e camundongos. A experimentação com animais sentinelas expostos a cama contaminada de camundongos experimentais é o método mais comum de monitoramento sanitário em instalações de animais. Embora a amostragem ambiental esteja sendo explorada e, em muitos casos, tenha sido implementada como alternativa, a amostragem do ar de exaustão de cabines ventiladas (CIVs) não é eficaz no controle de todos os patógenos que devem ser excluídos dos animais utilizados em ensaios. Entre os vírus murinos presentes em colônias de camundongos, destaca-se o Virus Diminuto do Camundongo (MVM). Este vírus pertence à família Parvoviridae e é considerado altamente prevalente em colônias de camundongos e um contaminante de alguns estoques de vírus e tumores transplantáveis. Como todos os parvovírus, a dependência da replicação do MVM em funções moduladas pela proliferação, diferenciação e transformação celular indica que o vírus requer células em divisão para se replicar. A replicação ocorre em vários órgãos, como pâncreas, intestino delgado, órgãos linfoides, fígado e rins. Este vírus é eliminado e transmitido pelas fezes e urina, onde pode persistir por várias semanas. Neste experimento, testes de PCR foram avaliados para a detecção de MVM em swabs colocados em cama contaminada em gaiolas sentinelas em uma VCI para monitoramento sanitário em colônias de camundongos de laboratório. Todas as amostras apresentaram resultado positivo para MVM. A implementação de swabs pode ser utilizada para substituir o uso e o abate de animais sentinelas e estabelecer um programa de monitoramento sanitário para patógenos prevalentes em biotérios de produção e pesquisa.

Palavras-chave: Reposição. Refinamento. Camundongos. Controle Sanitário.

RESUMEN

Los animales de laboratorio están expuestos a infectarse por una diversidad de agentes patógenos que son ampliamente reconocidos como factores adversos en las investigaciones biomédicas. De acuerdo con las normativas internacionales estos microorganismos deben estar ausentes en las colonias de ratas y ratones de experimentación. La experimentación con animales centinela expuestos a lechos contaminados de ratones de experimentación es el método más común para el control sanitario en instalaciones de animales. Si bien se está explorando el muestreo ambiental y, en muchos casos, se ha implementado como alternativa, el muestreo del aire de salida de gabinetes ventilados (IVC) no es eficaz en el control de todos los agentes patógenos que deben estar excluidos en los animales utilizados en los ensayos. Entre los virus murinos presentes en colonias de ratones se destaca el Virus Diminuto del Ratón (MVM) perteneciente a la Familia Parvoviridae considerado un virus de alta prevalencia en colonias de ratones y un contaminante de algunos stock de virus y de tumores trasplantables. Como todos los parvovirus, la dependencia de la replicación del MVM por funciones moduladas por la proliferación, diferenciación y transformación celular indica que el virus requiere de células en división para replicarse. La misma se produce en diversos órganos como el páncreas, intestino delgado, órganos linfoides, hígado y riñones. Este virus se libera y transmite a través de las heces y la orina, donde puede persistir durante varias semanas. En esta experiencia, se evaluaron pruebas de PCR para la detección de MVM en hisopos colocados sobre el lecho contaminado de cajas centinela en un IVC para el control sanitario en colonias de ratones de laboratorio. La totalidad de las muestras dieron

positivo para MVM. La implementación de hisopos puede utilizarse para reemplazar el uso y sacrificio de animales centinela y establecer un programa de control sanitario para patógenos prevalentes en bioterios de producción e investigación.

Palabras clave: Reemplazo. Refinamiento. Ratones. Control Sanitario.

1 INTRODUCTION

Laboratory animals are exposed to being infected by a variety of pathogenic agents that are widely recognized as adverse factors in biomedical research. According to international regulations, these microorganisms must be absent in rat colonies and rats of experimentation. In addition to the negative effects on the health of animals, these infections can cause changes in physiological functioning, causing interference in the results of research and exerting a negative influence on animal health (Compton & Riley, 2001; Blank et al., 2004).

Among the pathogenic agents that should be free of the animals used in experiments, we found the Among the murine Parvoviruses present in rat colonies, the Raton Miniature Virus (MVM) stands out, belonging to the Parvoviridae Family, considered a highly prevalent virus in rat colonies and a contaminant of some virus stocks and trasplantable tumors. Como todos los parvovirus, la dependencia de la replicación del MVM por funciones moduladas por la proliferación, diferenciación y transformación celular indica que el virus requiere de células en división para replicarse. It is also produced in several organs such as the pancreas, small intestine, lymphoid organs, liver and riñones. This virus is released and transmitted through the heces and the orina, where it can persist for several weeks. Healthy animals become infected in contact with the sick animal or with its heces and orina. El virus ingresa por via oronasal para luego diseminarse. Clinical signology is different in immunocompetent and immunocompetent animals. In general, in the first ones the clinical disease is not manifiesta unless they are subjected to a stress, but in the second ones it is more frequent to observe changes in their behavior and in their anatomophysiology. It can also reduce tumor growth rate directly or prevent tumor development, alter the modulation of tumor cell immune response, interfere with the selection of new transplants and cause a chemical reduction of tumors.

For all these reasons, the development of sensitive and precise diagnostic techniques allows us to know the efficiency of the sanitary barrier systems installed in the colonies, obtaining more representative data of the prevalence of this virus with the aim of knowing the magnitude of the infection and putting in practice the appropriate control measures. Currently, the diagnosis of MVM is carried out through the serological detection of antiviral antibodies in the population or by reaction in polymer chain (PCR) from specimens, organs or heces. The hemagglutination inhibition test (HAP) was the first technique used for the detection of antibodies (AC) but large viral antigen cantities are required. More recently, the most used serological techniques are the indirect immunofluorescence test (IFA) and the enzyme immunoassay (ELISA). The IFI technique is an easy-to-perform tool for a small size in the

samples of suero but ELISA is an alternative, sensible and specific automated technique commonly used for a large number of museums. The diagnosis of rat parvovirus (MPV) was found in the last few years, which should also be absent in rats used in experimentation, and the serological results were positive against MVM, due to the fact that most serological techniques present cross-action with MVM, and it is necessary to have complementary techniques that allow discriminating both viruses.

La posibilidad de diagnosticar infecciones en los animales de laboratorio depende de la sensibilidad de los métodos que se utilicen. Molecular tests are useful to demonstrate infections in rodent colonies through the detection of a specific DNA sequence of the pathogenic agent. The conventional Polymerase Chain Reaction (PCR) technique is widely used for the diagnosis of parvoviruses due to its high sensitivity and specificity in the health controls of laboratory rodent colonies (Charles Rivers, 2020).

Carrying out tests on all animals in a colony as part of a health surveillance program would result in time and cost endings. Therefore, for decades we have developed strategies to evaluate a smaller number of selected animals, which reflects the health status of the entire colony. Traditionally, the monitoring of the health of rodent colonies is carried out by means of specimens and centinela animals that have been exposed to the succinct bedding of animals in the colony. On the other hand, the collection of samples of extraction cameras in ventilated cabinets or backstage, followed by a PCR analysis, has become another promising method for health surveillance. However, environmental tests at the level of ventilated cabinets are not effective in all projects since some models of equipment are not suitable for the collection of particles from the extraction air. In this study, a colony of ratones of the C57BL/6J strain was used, which was previously demonstrated by a sanitary control of rutin, which was contaminated with MVM from samples of heces and sure, which were controlled by PCR and Indirect Immunofluorescence (IFA), respectively. After the same colony, the effectiveness of MVM detection was evaluated from a series of stories that were placed for three months in a centinela caja without animals, in which it was incorporated and accumulated samples of lecho sucio of the centinela cajas con ratones. Luego, with the hisopos placed and remaining in the caja centinela without animals, the bed sucia was mezcló o agitó semanalmente. In this way, the efficacy of this method for the detection of parvovirus by conventional PCR was determined. Asimism, through this molecular technique, the pools of heces collected from the centinela cajas con ratones were controlled to compare the results between the hisopos of the caja sin animales and those of the cajas con animales. This procedure could also be used for the sanitary control of agents prevalent in laboratory rats, which are transmitted through the litter of animals including viruses such as Coronavirus,

Norovirus, bacteria such as Helicobacter spp., Pasteurella pneumotropica (currently classified within the genus Rodentibacter) and endoparasites such as Syphacia obvelata. Based on the results of this experiment, the feasibility of establishing a health monitoring program for ratone colonies for these prevalent agents will be analyzed, eliminating the need to use centinell animals or animals from the colony itself (Shek, 2003; Shek et al 2005; Bauer et al., 2016; Blank et al., 2004; Bruin et al., 2016; Compton et al., 2004; Dubelko et al., 2018; Hanson et al., 2021).

2 GOAL

The objective of this experiment was to implement a pilot test for the sanitary control of laboratory ratone colonies for parvovirus (MVM) with good prospects of being implemented for other pathogens that are transmitted through the suicide of animal litter such as Coronavirus, Norovirus, Helicobacter spp, Rodentibacter pneumotropicus and Syphacia obvelata among others. This study was carried out using molecular PCR techniques in pools of heces collected from centinela cajas con animales and hisopos placed and agitated in samples of sucious lechos (of animals) in cajas centinela without animals, in order to compare the results of the control of the heces and of the hisopos. Este programa estoría un programa de controle sanitario para la colonia de ratones, eliminating the need to use centinela or own colonia animals.

3 METHODOLOGY

Adult conventional ratones (8 weeks old) of the C57BL/6J strain were used and housed in 4 cajas with 5 animals per caja, from a colony of a vivarium that showed positive results for Parvovirus (MVM) and other pathogens in successive health controls. A caja with 5 ratones free of specific pathogens (SPFs) of the C57BL/6J strain from the Laboratory of Experimental Animals (LAE) of the Fac was also used as a control sample. Cs. Veterinarias, UNLP. Three months after the beginning of the experiment, samples of conventional animals and SPF were taken. The test used was PCR was carried from samples of 5 tablets that were used by taking every 7 days of the milk for three months, coming from 4 cajas with 5 ratones in each one. In this experiment, 4 pools of heces from conventional animals and 5 stories from the suco of the cajas were controlled. Como muestra controle se analizó un pool de heces e hisopos agitados en lecho sucio de animales SPF. In this experience no animals were sacrificed. CEUA: Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the Faculty of Veterinary Sciences of the UNLP (License 122-6-22T).

4 FINDINGS

The results obtained through PCR techniques showed that all the liver pools of conventional ratones (4 samples) were positive for Parvovirus (MVM). Fecal samples of ratones control (SPF) were negative for Parvovirus (MVM). The results of the conventional centinel caja were all positive for Parvovirus (MVM). The results of the five hi-brains of the caja centinela control (SPF) were negative for the controlled agents.

5 CONCLUSIONS

The conclusions on the positive results obtained by controlling MVM show that the possibilities of those pathogenic agents that are effectively transmitted through the bed of the animals' bed bed can be detected from agitated stories in the beds used by the animals and dispense with the use of the same for rutin health controls. In this way, the implementation of these animals can be used to redefine the use and sacrifice of centinela animals in order to establish a health control program for pathogens prevalent in production and research vivariums, and to ensure that the results obtained in the investigations are free of uncontrolled variables.

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