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Sero-monitoring of viral pathogens affecting laboratory animals in a breeding colony

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Abstract

Health monitoring is an integral part in the management of laboratory animal breeding colonies. In this preliminary study, 66 samples from rats and 72 samples from mice were screened for antibody against 6 and 5 viral pathogens respectively. In case of Guinea Pig, 14 samples were screened for 3 viral pathogens. Among the pathogens examined in mice, incidence of Mouse Hepatitis Virus (94.44%) was very high followed by Minute Virus of Mice (20.83%) and in rats incidence of Sendai Virus (12.12%) was the highest followed by Sialodacryoadenitis Virus (7.58%) and Pneumonia Virus of Mice (6.06%). Guinea pig samples analysed were all negative for all the three pathogens. Overall health status of rat and guinea pig colonies is better than that of mice colony, due to the high prevalence of Mouse Hepatitis Virus and Minute Virus of Mice and their contagious nature, hence routine serological screening with stringent control measures are essential.

Keywords: Sero-monitoring, ELISA, Lab animals

Introduction

In the course of housing, laboratory animals are prone to develop various infections, mostly subclinical in nature, potentially influencing the outcome of the research [3, 6].

In addition, experimental procedures may also exacerbate subclinical infection to produce overt disease thereby increasing variability among experimental animals. Increased variability within the group may result in need of increased number of animals to achieve statistically significant result, further leading to misinterpretation and inconclusive results [12]. Consequently, microbiological status of both individual and population as a whole play a critical role in assessing quality and suitability of laboratory animal for experiments [13].

There is a need for routine microbiological screening of laboratory animals to understand the health status of the animals to implement control programme in infected colonies and to produce healthy animals for research and to prevent confounded experimental results [4].

The viral infections are difficult to manage in the breeding colony. Presence of viruses like Mouse hepatitis virus, Mouse parvo virus, Mouse minute virus and sendai virus have been reported to affecting the experimental results adversely [1, 2]. The presence of infection in laboratory animals can be detected by variety of methods [5]. Common serological method for the detection of prevalence of viral infections in laboratory animals is by using ELISA, which provides information about the microbiological status of laboratory animals [10].

Hence this study was conducted to understand the prevalence of commonly found viral infections in laboratory animals using ELISA in a breeding colony over a period of one year.

Materials and methods**Animal maintenance**

Animals included in this study were maintained at a breeding colony and was carried out after the approval of Institutional Animal Ethical Committee (IAEC), MVC, Chennai-07 and as per the guidelines of the CPCSEA, GOI.

Rats, mice and guinea pigs were maintained in polypropylene cages with Corn Cobb bedding material and supplied with *ad libitum* feed and water. Cages, bedding material and water cans are autoclaved and water is RO purified and autoclaved. Animals were maintained at room temperature 22±2°C and relative humidity 55±5% ventilated with centralized air conditioning by HVAC system.

Sample collection

Blood samples were collected from randomly selected weaned rat, mice and guinea pigs thrice in a year at the interval of four months. Animals were anaesthetized using 3% isoflurane. Blood samples were collected from anaesthetized rat and mice from the lateral tail vein. Guinea pigs were properly restrained and blood was collected by puncturing saphenous vein with 24G needle after shaving and applying liquid paraffin in the collection site. Clotted blood samples were centrifuged and the separated serum were stored as aliquots at -20°C used for ELISA.

Enzyme Linked Immunosorbent Assay

Serum samples were analyzed by Sandwich ELISA using the commercial kits (XpressBio Life Science Products, Thurmont, MD 21788, USA), for the presence or absence of antibody. Mice were screened for 5 viruses namely, Mouse Hepatitis Virus (MHV), Minute Virus of Mice (MVM), Pneumonia Virus of Mice (PVM), Mouse Sendai Virus (SeV) and Mouse Adeno Virus (MAdV).

While rats were screened for six viruses, Rat Adeno Virus (RAdV), Rat Parvo virus (RPV), Sialodacryoadenitis virus (SDAV), Sendai Virus (SeV), Pneumonia Virus of Mice (PVM) and Kilham's rat Virus (KRV) in rats, and Guinea pig for 3 viruses, Guinea Pig Adeno Virus (GAdV), Sendai virus (SeV) and Pneumonia Virus of Mice (PVM).

In brief, 100 µL each of 50 times diluted serum sample, the positive and negative control were added into the appropriate positive and negative antigen coated wells then covered and incubated for 45 minutes at 37°C. After washing five times

with wash solution, 100 µL of ready-to-use Peroxidase conjugate were added into each test well, covered and incubated for 45 minutes at 37°C. All the wells were added with 100 µL of ready to use ABTS peroxidase substrate after washing thoroughly. Plate was incubated at room temperature for 30 minutes. The extent of reactivity was extrapolated from the colorimetric reaction which was assessed by reading absorbance at 405 nm. The sample was considered positive if the difference in absorbance of the sample between the Positive Viral Antigen well and the Negative Control Antigen well was greater than or equal to 0.300.

Results and discussion

Throughout the year 152 samples were screened. Of which 66 samples from rats were screened for antibody against 6 viral pathogens, 72 samples from mice were screened for antibody against 5 viral pathogens and 14 samples from Guinea Pig for 3 viral pathogens. By examining the ELISA results for the pathogens mentioned above, 94.44% of mice were infected with one or more pathogens which were substantially higher than that of the rats (22.73%) and Guinea Pigs (0%).

Among the pathogens examined in mice (Table 1), incidence of MHV (94.44%) was very high followed by MVM (20.83%) and none of the samples were positive for PVM, SeV and MAdV (Table 1). Co-infection of MHV and MVM was evident from the result (20.83%) and MVM infection was never found alone. Incidence of MVM was higher in SAM (36.11%) than in BALB/c (5.56%), though such a difference was not evident in MHV infection.

Table 1: Incidence of viral pathogens in mice

Virus of mice	Swiss Albino Mice		BALB/c Mice		Overall % incidence
	Positive	% incidence	Positive	% incidence	
Mouse Hepatitis Virus*#	35/36	97.22	33/36	91.67	94.44
Mouse Adeno Virus	0/36	0.00	0/36	0.00	0.00
Pneumonia Virus Mice#	0/36	0.00	0/36	0.00	0.00
Sendai Virus#	0/36	0.00	0/36	0.00	0.00
Minute Virus of Mice*	13/36	36.11	2/36	5.56	20.83

*All the samples positive for minute virus of mice were also positive for Mouse Hepatitis Virus

indicates RNA virus

The most prevalent virus in India is MHV followed by MMV^[8], which is concurrent with the present finding. However the prevalence rate of MHV in the present study was very high. Wherein the overall prevalence rate in India accounts less than 50%, which included both public and private organizations^[8]. But it is similar with prevalence rate in mice colony TIFR, Mumbai, India^[6]. The high prevalence rate of MHV may be due to the highly contagious nature of the virus

^[9].

In rats (table 2), incidence of SeV (12.12%) was the highest followed by SDAV (7.58%), PVM (6.06%) and none of the samples were positive for KRV, RAdV and RPV. Guinea pig samples analyzed were all negative for all the three pathogens (Table 2). Like in mice, co-infection of SeV and SDAV was evident in two samples.

Table 2: Incidence of viral pathogens in Rats and Guinea Pigs

Virus of rats	Wistar Rats		Guinea Pigs	
	Positive	% incidence	Positive	% incidence
Rat Adeno Virus	0/66	0.00	-	-
Rat Corona Virus / SDAV*#	5/66	7.58	-	-
Kilham's Rat Virus	0/66	0.00	-	-
Rat Parvo Virus	0/66	0.00	-	-
Sendai Virus*#	8/66	12.12	0/14	0.00
Pneumonia Virus Mice#	4/66	6.06	0/14	0.00
Guinea Pig Adeno Virus	-	-	0/14	0.00

*Two of the samples were positive for both SeV and SDAV

indicates RNA virus

SeV is not prevalent in the mouse colony and it is low in rat colony which is similar to the findings of Kohale & Raut (2013) [6]; C-T- Liang *et al* (2009) [7] in Taiwan and Schoondermark-van de Ven *et al* (2006) [11] in western Europe, who had reported that there is decline in prevalence of sendai virus in recent years due to health monitoring along with improved knowledge in laboratory animal science and husbandry.

In rats, overall prevalence of virus is very low compared to mice and there is no prevalence of KRV, RAdV and RPV indicating better health status in rat colony for the set of viruses analysed. On examining the results further, it is evident that the viruses found positive were all RNA virus except MVM which is DNA virus. In addition, in spite of the fact that samples from all the three species were from same facility and were screened for both SeV and PVM, only rat samples were found to be positive.

Conclusion

Routine Health monitoring is a mandate in every breeding colony. As per this preliminary study, overall health status of rat and guinea pig colonies is better than that of mice colony. Despite the high incidence of MHV and MVM in mice colony, clinical infection was not evident in any of the animal, which imposes threat to the health status of mice colony due to sustenance of infection, hence routine screening with stringent control measures are regarded essential.

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