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Effect of Cross-Fostering on a Variety of Viral Agents in Conventionally Bred Rats

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Abstract

We aimed to compare the serological profiles of the pups that were housed with their biological mothers and the ones which were transferred to foster-dams after birth, with regard to certain viral agents. The day of delivery for the pregnant rat is recognized as the postnatal day 0 (P0). The pups born from Group 1 [P(0), P(21), P(60)] pregnant rats were housed with their biological mothers. In Group 2 [P(1), P(21), P(60)], however, P(1) consisted of pups housed with their biological mother for 24 hours while P(21) and P(60) consisted of pups transferred from their biological mother immediately at birth. Antibody response against Lymphocytic Choriomeningitis Virus (LCMV), Rat Parvo Virus (RPV), Sendai Virus (SV) and Toolan's H1 viruses was not detected in dams and pups of all the groups, whereas high rate (45%) of antibody positivity was observed in response to Kilham' Rat Virus (KRV). No statistically significant difference was determined between the pups left with their biological mother and with the foster mothers, except for Murine Adeno Virus type 1, type 2 (MAD 1 and 2), Reovirus type 3 (REO-3) and Theiler's Murine Encephalomyelitis Virus (TMEV). It was conspicuous in terms of REO-3 that for the pups it was essential to stay with their biological mothers and nursed during the first 24 hours. As a result, foster mother usage in breeding laboratory animals is found to be beneficial against a variety of viral agents for the pups particularly in the later period of their lives and we suggest to continue this implementation in routine laboratory practice whenever necessary.

Keywords: Cross-fostering; Rat; Microbiological monitoring; Viral agents

Introduction

Experimental animals are widely used in many fields such as infectious, immunologic and endocrinological diseases and cancer ethiopathogenesis, and they provide important data for the protection of human health and quality of life. It is essential for the experimental research animals (laboratory animals) to be

healthy and showing no signs of any clinical disease as well as carrying no infectious agent in latent form. Microbiological agents are known to have considerable impact on the health status of laboratory animals and on the outcomes of the experimental studies. Physiological, biochemical and endocrinological parameters of sick and healthy animals are normally different from each other so these may affect the outcomes of the experimental studies [1-3].

Rats are among the most commonly used animals for scientific purposes [4]. Rat pups are born hairless and with eyes closed, after a gestation period of 20-22 days. They acquire passive immunity from their mothers through placenta and colostrum which protect them against various infectious agents in the first few days of their lives [5,6]. Therefore it is very important to keep rat pups with their biological mother's right after birth and allow for nursing. Fostering is routinely used in breeding of both laboratory and farm animals, and it is important for supporting pups whose dams die in lactation period or while giving birth to a bigger pup.

In laboratory animal science, protective medicine is fundamental. It is important to observe the symptoms and determine any disease and take precautions before it spreads to the colony. It is also important to have information about the microbiological status of the experimental animals and their environment with a view to animal welfare too, in addition to reliable scientific results. For this purpose, the Federation of European Laboratory Animal Science Associations (FELASA) is regularly revising its recommendations on monitoring the health of the production colonies or experimental units [7].

In this study, we investigated the effects of cross-fostering on microbiological monitoring of conventionally bred rats. We aimed to determine the serological profiles of the pups that were either housed with their biological mothers or transferred to foster-mothers and compare these profiles against certain viral agents that can be seen in the laboratory animal facilities and as well as to demonstrate potential differences between them.

Materials and Methods

Animals

Conventionally bred and housed rats were obtained from the Multidisciplinary Animal Laboratory of Dokuz Eylul University (DEU). The laboratory has authorization within the framework of the regulation issued by Food, Agriculture and Livestock Ministry framework since 2008 for the production, use and supply of animal species such as mice, rats, guinea pigs and rabbits. The study was approved by the Ethics Committee of the Research of Laboratory Animals, DEU Medical School. The rats were housed in hygienic makrolon cages, with ad libitum-feeding, in air-conditioned rooms with 12-hour light-dark cycle. The protocols complied with the standards in the Guide for the Care and Use of Laboratory Animals, prepared by the National Academy Press, National Research Council [8].

Study groups

Nonsibling and virgin Wistar albino female rats (200-230g) (n=6) were mated in separate cages. (Power analysis was performed to use the minimum number of animals.) Following mating, positively pregnant females were marked and kept in separate cages under standard conditions until delivery. The pregnant females comprised of randomly grouped and blood samples were collected to determine their baseline serological status. All the females gave birth in two days. First four females were taken to biological mother group, other two females taken to foster mother group. Pups of foster mothers were taken to stock colony. The day of delivery was recognized as the postnatal day 0 P(0). Considering postnatal period, Group 1 was subdivided into P(0), P(21), P(60) subgroups and Group 2 into P(1), P(21), P(60) subgroups. Groups and characteristics are summarized in **Table 1**. The dams and pups in all groups were weighed and recorded. In case the mother gave birth to more than seven litters, then these were excluded from the study and transferred to breeding colony. We made no discrimination between female and male pups born in the study. After completion of the experimental protocol described in **Table 1**, dams and pups in each group were sacrificed by cervical dislocation and then the blood samples were collected by cardiac exsanguinations [9]. Sera were separated and stored at -80°C until the day of testing.

Determination of viral antibodies by ELISA method

On the test day, rat serum samples were taken out of the freezer at -80°C and left to dissolve at +4°C. The levels of antibodies against Kilham's Rat Virus (KRV), Lymphocytic Choriomeningitis Virus (LCMV), Murine Adeno Virus Type 1, Type 2 (MAD 1 and 2), Rat Parvo Virus (RPV), Reovirus Type 3 (REO-3), Sendai Virus (SV), Sialodacryoadenitis Virus / Rat Coronavirus (SDAV/RCV), Toolan's Virus (H1), Theiler's Murine Encephalomyelitis Virus (TMEV) were tested by ELISA method in accordance with the manufacturer's recommendations (SMART, Biotech Trading Partners, CA-USA). The optical density values of the plates were measured by an ELISA microplate reader device (Thermo Electron Corporation, Varioskan Flash, USA) at

450/620-650 nm. Index value was calculated for the reagent control, negative control, and each serum sample. All tests were run twice by different researchers in different periods.

Statistical analysis

All statistical procedures were performed by SPSS 15.0 software, Mann-Whitney U test was used in comparing the (medians) of two groups. p<0.05 was accepted for statistical significance. Articles were evaluated using a checklist derived from the Animal Research: Reporting of *in vivo* Experiments (ARRIVE) guidelines [10].

Ethical statement

This study was approved by the DEU Local Ethics Committee (48/2012) and Three-Rs/3R principle was preserved without compromise throughout the study. The assessment was performed on animals in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences [8]. All the researchers of this study have the Certificate on Experimental Animal Use and the author in charge also holds FELESA C Category Certificate on Experimental Animal Use (Utrecht University, The Netherlands).

Results

In our study, antibody response was not detected against LCMV, RPV, SV and Toolan's H1 virus neither in dams nor in pups of group 1 and group 2 (**Figure 1**). A high level of KRV antibody positivity (45%) attracts attention in all groups. High antibody levels detected in the young pups after delivery suggests intrauterine transmission of antibodies from the mothers; whereas these levels were observed to decrease after day 21 and eventually after day 60 they reach the level that only indicates previous infections. Just a similar situation is also notable for RCV.

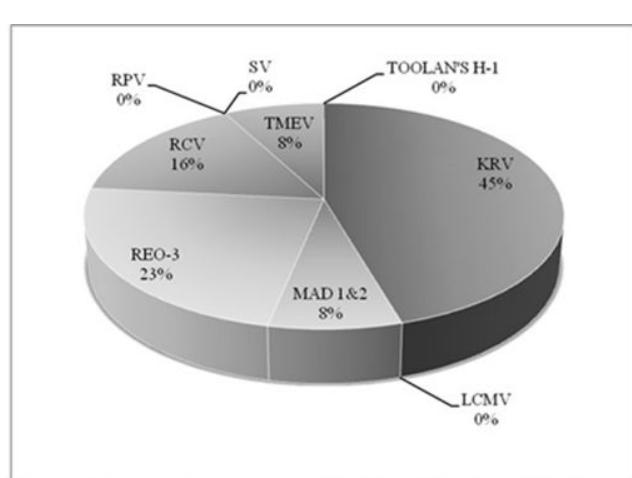


Figure 1: Antibody positivity against KRV, LCMV, MAD 1 and 2, RPV, REO-3, SV, SDAV/RCV, H1 and TMEV in all groups (dams and pups).

As for the agents other than MAD 1 and 2, REO-3 and TMEV, there was no statistically significant difference between fostered-pups and those left with their biological mothers ($p>0.05$). For REO-3 virus, it was important for the pups to stay with their biological mothers and nursed by them in first 24 hours (**Figures 2 and 3**).

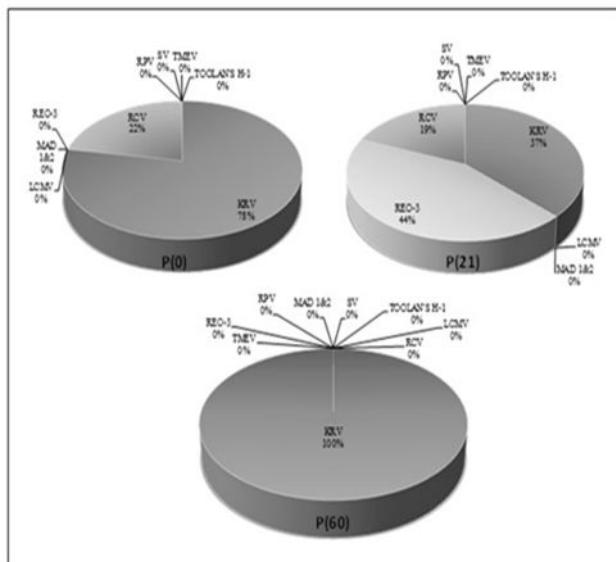


Figure 2: Antibody positivity against KRV, LCMV, MAD 1 and 2, RPV, REO-3, SV, SDAV/RCV, H1 and TMEV in group 1 pups.

Transmission of REO-3 virus antibodies from dams to pups was found significantly higher in Group 2 P(1) which were hosted for 24 hours with their biological mothers when compared with those in group 1 P(0) which were sacrificed immediately after birth ($p=0.006$). Antibody positivity against MAD 1 and 2 and TMEV infection was found only in the dams and pups of group 2 P(21) and not observed in the other groups (**Figure 3**). Serological profiles of all experimental groups are shown in **Table 1**.

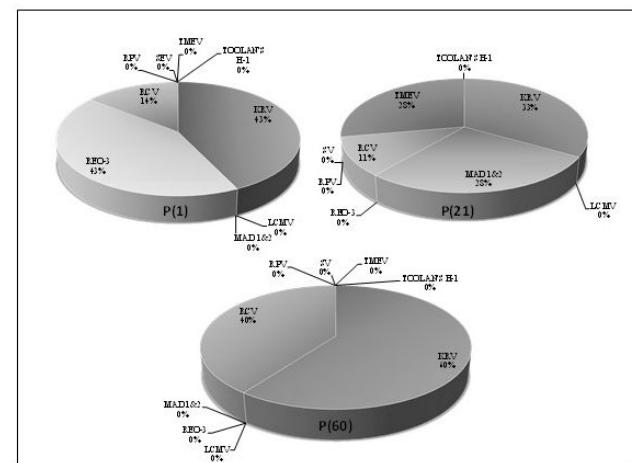


Figure 3: Antibody positivity against KRV, LCMV, MAD 1 and 2, RPV, REO-3, SV, SDAV/RCV, H1 and TMEV in group 2 pups.

Table 1: Study groups and features.

Groups	Features	Reason of formation
Group 1, P(0)	Dams and pups scarified just after birth	Transplacental antibody transmission
Group 1, P(21)	Pups were housed with their biological mother and breastfed for 21 days after birth	Transplacental transmission and antibody transmission from biological mother via breastfeeding
Group 1, P(60)	Pups were housed with their biological mother and breastfed for 21 days after birth then fed ad libitum by standard pellet diet until day 60.	Transplacental transmission, antibody transmission from biological mother via breastfeeding, micro and macro environmental effects
Group 2, P(1)	Pups were housed with their biological mother for 24 hours after birth	Transplacental transmission and antibody transmission from biological mother via breastfeeding during 24 hours
Group 2, P(21)	Pups transferred to foster-dam just after birth and breastfed by foster-mother for 21 days	Transplacental transmission, antibody transmission from foster-dam via breastfeeding
Group 2, P(60)	Pups were housed and breastfed by foster-dam for 21 days then fed ad libitum by standard pellet diet until day 60	Transplacental transmission, antibody transmission from foster-dam via breastfeeding, micro and macro environmental effects

Discussion

In recent years, a significant increase is apparent in the variety and quality of laboratory animals internationally, regarding the need in scientific research area. Microbiological monitoring is also improved along with the increased variety of genetically defined animal models. The researchers, pointing out the necessity for high security measures on this matter, indicate that we need reliable guide sources and programmed animal experiments for pathogen detection [11]. Identifying common pathogens and taking due preventive precautions constitute

fundamental steps of microbiological monitoring implemented on laboratory animals. Although detecting high-risk pathogens is the priority of microbiological monitoring protocols, our main objective is tracking the incidence trends of infectious agents over years and questioning the sufficiency of the standard conditions in the colony [12]. Information on the microbiological status of the experimental animals (laboratory animal) and their environment will be the key to achieve repeatable and reliable access to scientific data by using the minimum number of animals (Reducement) and to improve animal welfare (Refinement).

Cross-fostering practice is commonly utilized in breeding of laboratory animals. This is an important practice for the survival of the pups whose dams died in lactation period or while giving birth to a bigger pup. Passive immune agents that protect pups against various infectious agents in the first days of life are acquired from their mothers through the placenta and colostrum [5,6]. Therefore staying with their mothers after birth and nursing is very important for the offspring. In our study, serological profiles against certain viral agents were compared between pups housed with their biological mothers or transferred to foster-mothers' cage immediately after birth and the effects of foster-mother usage were evaluated. At this point, FELESA recommendations on microbiological monitoring of production colonies and research units were adhered and viral agents as well as study methods were determined accordingly [13]. All serological tests in this study were performed by ELISA method. ELISA is frequently preferred in routine microbiological monitoring of the laboratory rats and mice, for being easily applicable and objectively assessable. It has been indicated in some studies that the serum samples found to be positive by ELISA should be confirmed by IFAT. However, further IFAT confirmation could not be performed in this study because serum samples were not enough for further analysis after testing for antibodies against nine different viruses.

Maternal antibodies are known to protect pups against environmental pathogens. Transmission of antibodies generally occurs through placenta or lactation from infected or naturally immunized mothers to pups.⁶ In the study by Offit et al. [14] female mice were parenterally inoculated with non-infectious Rota virus (RRV) and observed that they developed specific antibodies over time. These authors demonstrated the presence of immune response against RRV-induced gastroenteritis in the mice born from these dams. This study showed that immune system factors are maternally transferred from such passively immunized dams to their pups.

Arthwohl et al. [15] however, investigated the effects of fostering in mice against Murine Norovirus, Helicobacter spp. and Murine Hepatitis Virus and the level of protection against these agents after two years. Fostered-pups showed no difference in the incidence of these infectious agents. Also in our study, the fostered-pups or those left with their biological mothers displayed no statistically significant difference with respect to the agents other than MAD 1 and 2, REO-3 and TMEV ($p>0.05$). REO-3 virus is known to have direct contact transmission by direct contact but with extremely low contagiousness among laboratory rodents. Transmission from infected mothers or cage materials is reported to be limited [16]. In our study, REO-3 antibody positivity was found to be 23% as shown in the **Figure 1**, when all groups were assessed together. When individual sub-groups were assessed separately, antibody positivity against REO-3 was detected in both birth- and foster-mothers but not in any pups of group 1 P(0), group 1 P(60) and group 2 P(60). In the pups, antibody transition during pregnancy was not detected; furthermore REO-3 infection due to direct contact with environmental factors was not determined in later days of life, which supports that transition is rare and random. On the other hand, housing the pups and nursing by their biological mothers for the first 24 hours appears to be significant

in terms of REO-3. Thus, pups can be protected during the first moments of life against this virus which is transmitted through various environmental factors, secretions and direct contact.

In our study, antibody positivity against MAD 1 and 2 and TMEV was determined in sera of dams and pups of group 2 P(21) only, but not in other groups (**Figure 3**). Both viral agents show transmission via body secretions, direct contact or intestinal tract. Determining antibody positivity only in this group suggested that foster-mothers acquired both viruses from a source and transferred to fostered-pups through nursing. Considering serum antibody levels of these fostered-pups which were housed with foster-mothers for 21 days after birth, it may be assumed/anticipated that serum may become negative over time. The fact that the antibody positivity against both viral agents was around 8% when all groups were assessed, it is supporting their low incidence among laboratory rats [17,18]. Based on these data, we can claim these viruses are seen very seldom in our facility; also this foster-mother might have acquired these viruses from the environment and transferred to pups through nursing.

In the literature, LCMV and Toolan's H1 virus infections in the laboratory rat colonies are reported to be rare [19]. LCMV, Toolan's H1 and RPV was not identified in a study conducted by the collaboration of various research centers, universities and biotechnology companies that bred and maintained laboratory mice and rats by conventional methods between 2004-2009 in Australia [20]. Antibodies were not detected in any dams or pups in our study too, against Toolan's H1 and LCMV, which is a viral zoonotic agent. Our study is consistent with the above mentioned study, which, in this respect, was one of the most comprehensive studies of the recent years.

Sendai virus is one of the most important infectious agents for laboratory rats and mice. Sendai virus is highly infectious and spreads via direct contact as well as inhalation [17,20]. It is a remarkable finding that no antibody positivity against SV and thereby no previous SV infection was determined in any of our study groups. This may indicate housing and hygiene conditions and care practices in our breeding and stock units are adequate enough at least against this agent which can be transmitted in a colony via inhalation.

Serum samples of 392 young rats were obtained from 20 different units in Argentina, engaged in conventional breeding of laboratory animals between 2006 and 2008. The incidence of Kilham Rat Virus (KRV) was reported to be 51.8% (203/392) in these sera. In the same period, KRV infection was not determined in the units engaged in SPF breeding as well [21]. The highest antibody positivity in our study was against KRV (45%) in all groups. High levels of antibody positivity determined in young pups after delivery suggested intrauterine transmission whereas these levels decreased from the 21st day on. Furthermore, antibody positivity related to previous infections was noted from the 60th day on. This proves that KRV was predominant in our breeding and stock colony and this viral infection may emerge in utero and from the first moments of life and immunize the rats. Appropriate disinfection particularly of base materials and cages should be considered important and due measures should be taken against this agent which can be

transmitted by secretions such as urine, feces, vomit, and milk. Because, KRV was determined to be negative in conventionally bred rats in a five-year study carried out by McInnes et al. [18] in Australia, which is an indication that such measures would help. Zenner et al. [19] have also reported that the incidence of infectious agents in the colony decreased through regularly conducted monitoring and hygiene protocols.

Conclusion

It is obvious that routine microbiological monitoring is a significant requirement especially in the facilities engaged in conventional breeding and maintaining. Initially, this situation can be perceived as an unnecessary or futile work in terms of manpower and financial burden. However, it will improve the quality of the colonies and the work carried out with these animals, and also it will prevent risking the whole colony in case of any infection. On the other hand, we have concluded that foster-mother usage would protect pups against a variety of viral agents in later periods of life and hence this practice should be considered to continue whenever required.

Conflict of Interest

None declared.

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