Study of zoonotic bacteria in conventional laboratory mice breeding colonies

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Abstract: **To investigate the status of conventional laboratory mice colonies for zoonotic bacteria, three mice colonies, NIH, NMRI and Balb/c strains of Razi institute were studied for five zoonotic bacteria including *Pasteurella pneumotropica*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Streptobacillus moniliformis*, *Streptococcus Pneumonia*. Study populations were 74 mice of premature, mature and post mature ages. Same numbers of male and female animals were allocated in a random fashion from each 3 strains of mice. Two hundred and ninety six specimens of nasopharynx (n=156)),cecum (n=70) and liver (n=70) were taken from 74 animals and cultured for bacteriological tests. Results showed that none of tested specimens, infected by mentioned zoonotic bacteria, however in 78% (231/296) of mice, commonly nonpathogenic bacteria, mainly *Escherichia coli*, *Bacillus sp*, Streptococcus other than *Streptobacillus moniliformis*, and *Entrobacter aerogenes* were observed. The results of study recommend use of these animals have no human risk or research interferences for mentioned zoonotic bacteria. However like other conventional laboratory animals, precaution and personnel's safeties must be considered while using in researches where the existing bacteria may interfere the results.**

Key words: zoonoses, conventional mouse, bacteriological test, breeding colony.

Introduction

Laboratory animals microbiological monitoring is a vital importance decreasing the risk of zoonoses and adding to the reliability and reproducibility of research data (Weisbroth, 1979). Different genetic background, breeding system and environmental conditions may result into different health status in the animals (Christensen *et al*., 1997; Cunliffe-Beamer *et al*., 1994). Any changes in these factors may alter host physiology and the animal that appear normal and healthy may become unsuitable as research subjects due to the unobservable but significant local or systemic effect of infectious agents with which they may be infected. The latent infection of laboratory animals may show no overt disease but research results may be compromised through subtle physiological, biochemical or histological changes however they may threaten researchers and workers health as well (Baker, 1998; Cunliffe-Beamer *et al*., 1994; Rehbinder *et al*., 1996). In laboratory animals, infection more frequently is asymptomatic with carriers who develop overt disease when stressed by shipping or experimental manipulation. There are a number of pathogens associated with commonly used laboratory animal species which may be endemic in some conventional rodent colonies. Conventional animals are those that are not raised or held under any particular barrier system and their health status is

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generally unknown (O'Brien, 1993). For health monitoring of laboratory animals there are three major groups of micro -organisms (Kraft *et al*., 1994; Rehbinder *et al*., 1996; Nicklas *et al*., 2002). The first priority are zoonotic organisms which are naturally transmitted between vertebrate and man (World Health Organization). The second group of microorganisms to be screened are those that are latent and may cause clinical diseases when animal is subjected to experimental stress. Third group to be considered are those that may interfere with experiment, whether clinically evident or only sub-clinical at the time of experiment. The mode of transmission of zoonotic agents is by feces, urine, saliva, blood or milk via aerosol, oral, contact with bleeding or animals, etc. The probability of disease transmission from animals to man is influenced by several factors. Length of time the animal is infective, length of the incubation period in animals, stability of the agent, population density of the animals in the colony, husbandry practices, maintenance procedures and control of wild rodents and insects, virulence of the agent and route of transmission. There are 25 types of bacteria recognized as zoonoses in small laboratory animals (Christensen *et al*., 1997; Cunliffe-Beamer *et al*., 1994; Ganaway, 1982; O'Brien, 1993). To reduce the range and prevalence of pathogens found in lab animals and also to gain reliable and repeatable experimental results, microbiological definition of the animals must be known to the researchers and workers. At first phase in this study five of important zoonotic bacteria's of laboratory mice including *Pasteurella pneumotropica*, *Salmonella typhymurium*, *Salmonella enteritidis*, *Streptobacillus moniliformis*, *Streptococcus pneumonia* were considered for monitoring of breeding colonies according to the FELASA recommendations (Kraft *et al*., 1994; Rehbinder *et al*., 1996; Nicklas *et al*., 2002). We believe this program will benefit the entire laboratory animal and biomedical research communities, and joint efforts between diagnostic laboratories, vaccine producing departments, and laboratory animal department can achieve the common goal of healthy animals being used in biomedical research in Iran. A comprehensive program such as this currently does not exist in the country.

Materials and Methods

Seventy four conventional laboratory male and female mice, thirty NIH, twenty NMRI and twenty four Balb/c strain of 3-4, 6-8 and 25 weeks old were randomly selected from breeding colonies of Razi Vaccine & Serum Research Institute. Animals were housed at temperature of 22±2 ºC, humidity 50±5%, air exchange 10-12 times per hour, fed with standard diet (made by Razi vaccine & serum research institute located in Hesarak, Karaj, Iran), and tap water was given ad lib. Where suspected prevalence rate of an infection in our breeding unit is considered 30% with confidence level of 95%, and following assumptions were considered:

1. The breeding unit is here understood as self-contained microbiological entity in which animals are bred in each unit one species (such as mouse or rat) is kept.
2. Both sexes are infected at the same rate.
3. Population size >100 animals.
4. Random sampling.
5. Random distribution of infection.

The sample size is calculated from the following formula (Nicklas *et al*., 2002) and 10 animals per breeding unit for each species is used:

$$\text{Sample size} = \frac{\text{Log } 0.05}{\text{Log N}}$$

N = Percentage of non-infected animals, 0.05 = 95% confidence level

A total of two hundred and ninety six specimens of nasopharynx (n=156)),cecum (n=70) and liver (n=70) of 74 anesthetized animals were collected under sterile conditions. For detection of bacteria, ceacal samples were cultured on Tetrathionate Broth and samples from nasopharynx and liver were cultured on TSB (Trypticase Soy Broth). Tubes were incubated at 37 $^{\circ}$C, 5 % CO_2 for 24-48 h. Samples were further cultured on SS (Salmonella Shigella Agar), BA (Blood Agar), TSA (Tryptic Soy Agar), EMB Agar, MacConkey Agar and reincubated for 24-48 h. Colonies were identified based on colony morphology, in case colonies with concaved centers were observed, gram staining tests were performed to

Table 1: Bacterial status of male and female conventional mice of NIH, NMRI and Balb/c breeding colonies. Footnotes: W=week; F=female; M=male.

Bacteria	NIH						NMRI						Balb/c						Total (%)	
	3-4 (w)		6-8 (w)		25 (w)		3-4 (w)		6-8 (w)		25 (w)		3-4 (w)		6-8 (w)		25 (w)			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Streptobacillus pneumonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)
Pasteurella pneumotropica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)
Salmonella typhimurium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)
Salmonella enteritidis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)
Streptobacillus moniliformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)
Escherichia coli	2	6	4	1	0	1	5	3	1	1	2	0	2	2	0	1	1	2	17 (14.7)	17 (14.8)
Bacillus sp	3	4	6	6	3	5	0	0	0	0	0	1	0	0	1	3	0	1	13 (11.2)	20 (17.4)
Staphylococcus sp	1	0	1	1	4	1	0	0	0	0	0	0	1	0	0	0	0	0	7 (6.0)	2 (1.7)
Entrobacter aerogenes	4	4	3	4	1	0	0	2	0	0	1	0	4	2	1	1	4	0	18 (15.5)	13 (11.3)
Proteus sp	3	1	2	2	2	0	1	0	2	1	2	3	0	0	0	0	1	1	13 (11.2)	8 (7.0)
Streptococcus sp	1	1	0	0	3	4	5	6	2	2	4	5	4	6	7	5	5	4	31 (26.7)	33 (28.7)
Klebsiella sp	0	0	0	0	1	2	1	0	1	1	0	2	1	1	0	0	0	0	4 (3.4)	6 (5.2)
Providencea sp	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0 (0.0)	2 (1.7)
Bacillus+Streptococcus	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1 (0.9)	1 (0.9)
Bacillus+ Staphylococcus	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1 (0.9)	1 (0.9)
Bacillus +E coli	3	2	0	2	0	0	0	0	0	0	0	0	0	0	2	0	0	1	5 (4.3)	5 (4.3)
Bacillus+Entrobacter	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2 (1.7)	1 (0.9)
Bacilusl+Proteus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (0.9)	0 (0.0)
E coli+Providencea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0 (0.0)	1 (0.9)
E coli +Klebsiella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1 (0.9)	0 (0.0)
E coli+Entrobacter	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0 (0.0)	2 (1.7)
E coli +Proteus	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	2 (1.7)	2 (1.7)
Streptococcus+Entrobacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0 (0.0)	1 (0.9)
Total	17	18	18	18	15	14	12	12	6	6	12	12	12	12	12	12	12	11	116 (100.0)	115 (100.0)
No growth	3	2	1	2	6	6	4	4	2	2	4	4	4	4	4	4	4	5	32 (21.6)	33 (22.3)
Sum total	20	20	19	20	21	20	16	16	8	8	16	16	16	16	16	16	16	16	148 (100.0)	148 (100.0)

detect the bacterium. Further differentiation is performed by using biochemical tests. (Feder *et al*., 2001; Gupta and Briski, 2004).

Results

In two hundred ninety six samples that were cultured from nasopharynx, cecum and liver of mice, *Streptococcus pneumonica*, *Pasteurella pneumotropica*, Salmonella *Spp*, *Streptobacillus moniliformis* were not seen (0/296) in all samples. However in 78% of samples (231/296) infection to one or two bacteria was observed.

In 14.7% (34/231) of cases *Escherichia coli* was observed in nasopharynx (16/34), cecum (16/34) and liver (2/34). 58.8% (20/34) of them was present at age of 3-4 week old mice, where 41.2% (14/34) were present at age of 6-8 and 25 week mice. There was no significant strain difference. Thirty three cases (33/231) *Bacillus spp* was mainly detected from NIH (27/31), one case (1/33) in NMRI and five case (5/33) in Balb/c strain mice. Nine (9/231) *Staphylococci spp* were observed in samples, 88.9% (8/9) were seen in NIH, 0% (0/9) in NMRI and 11.1% (1/9) case in Balb/c strain. 13.4% (31/231) *Entrobacter aerogenes* was observed, so 51.6% (16/31) in 3-4 week age, 29% (9/31) in 6-8 week age and 19.4%

(6/31) in 25 week age groups.

Twenty one (21/231) Proteus sp were mainly found in ceacal samples. Eighteen cases were proteus sp, two proteus vulgaris and one morganella morganii type Ten of them in NIH, nine in NMRI and two in 25 weeks old Balb/c strain.Sixty four (64/231) Streptococci sp were found, 85.9% (55/64) mutans type in nasopharynx of three strain mice (thirty one in Balb/c, twenty in NMRI & four in NIH), 11% (7/64) streptococci sp and 3.1% (2/64) were agalactia type. Three cases of streptococci sp were found in NIH, four in NMRI and none in Balb/c strain. Where two streptococcus agalactia were found in nasopharynx of 25 old NIH and none in NMRI and Balb/c strain. Ten (10/231) Klebsiella sp were observed, three in nasopharynx and cecal samples of 25 week old NIH, five in NMRI of all three age group and two in cecum of 3-4 week old Balb/c strain mice. Two (2/231) Providencea sp were detected in 25 week aged groups of NIH and Balb/c strains. In 11.7% (27/231) of samples coexistence of two bacteria were detected as shown in table1. There was no any bacterial growth in 22% (65/296) of samples, so twenty cases in NIH, twenty cases in NMRI and twenty five cases in Balb/c strain mice. Most of liver samples were bacterial free.

In overall there was no significant difference between total infected samples of male and female animals (27.6% (116/148) versus 28.7% (33/148) respectively), between three age groups and also between of three strain mice groups with each other ($p>0.05$) table 1.

Discussion

In this study we investigated the bacterial status of breeding colonies of conventional raised mice. The methods used are according to recommendation of FELASA for breeding colonies (Kraft *et al*., 1994; Nicklas *et al*., 2002). Simply relying on the absence of clinical disease in laboratory animals is not sufficient. The microorganism data is a part of animal specification and should therefore be evaluated for their influence on the results of experiments. The data should be made available to those researchers using the animals. The designation of unit (non barrier, barrier, isolator) should be included in information of animal report (Nicklas *et al*., 2002), any change in the unit system may change the animals health status. Identification of all strains present within the unit and date of issue of the report is also important to be known. (Rehbinder *et al*., 1996; Nicklas *et al*., 2002).

The Streptobacillus pneumonia, *Pasteurella pneumotropica*, Salmonella sp and *Streptobacillus moniliformis* are reported as zoonotics in laboratory mice that are included in the list of microorganisms to be monitored (Baker, 1998; Christensen *et al*., 1997; Cunliffe-Beamer *et al*., 1994; O'Brien, 1993).These zoonotic bacteria were absent in the studied strains of mice of razi institute, that could be because of implementing program tailored to the facilities design and management to elevate standards of animals care. *Escherichia coli*, *Bacillus sp*, Staphylococcus sp and Entrobacter sp are bacteria that are reported to be present in mice colonies (Baker, 1998; Cunliffe-Beamer *et al*., 1994; Ganaway, 1982; O'Brien, 1993; Weisbroth, 1979; Yanabe *et al*., 2001).In early reports E.coli appears to be of little significant as a pathogen for mice. However in a survey of seven strains of conventionally raised mice (C57BL/6cr, DBA/2cr, BDF1, C3H/Hej, BRVS/srcr, and DBA/2cr), E.coli was isolated from feces of 81% of 382 adult mice. Staphylococcus sp are usually considered part of resident microflora and are occasionally found on routine culture of nasal passages of healthy mice. It is also reported that, mice may harbor *Streptococcus sp* in their upper respiratory tracts without signs of illness. *Klebsiella pneumoniae* which is a gram-negative bacterium, normally inhabiting the intestinal tract of mice and rats and it considered as an opportunistic pathogen (National Research Council, 1991). *Streptococcus spp* is a gram-positive diplococcus, commonly found in laboratory rodent colonies. Natural infections of respiratory system of laboratory rats and mice with *Streptococcus spp* have been shown to alter hepatic metabolism, levels of biochemical in serum, blood pH and electrolytes, thyroid function, and respiratory parameters (Baker, 1998).

The results of study recommend use of the three strain of laboratory mice have no human risk or

research interferences for mentioned zoonotic bacteria. However, while using conventional laboratory animals, precaution and personnel's safeties must be considered.

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مطالعه باکتری های زئونوز در کلنی های موش های آزمایشگاهی متعارف

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به منظور بررسی باکتری های زئونوز در کلنی موش های آزمایشگاهی متعارف، در این تحقیق پنج باکتری زئونوز شامل *pneumonia Salmonella enteritidis, Streptobacillus moniliformis ,Pasteurella pneumotropica, and Streptococcus Salmonella typhimurium,* در کلنی موش های متعارف موسسه رازی در سه نژاد NIH، NMRI و Balb/c مورد بررسی قرار گرفتند. جمیعت مورد مطالعه ۷۴ سر موش، در سنین قبل از بلوغ، بلوغ و پس از بلوغ بودند. این حیوانات به تعداد مساوی از جنس نر و ماده بطور تصادفی از هر سه نژاد انتخاب شدند. تعداد ۲۹۶ نمونه از قسمت های نازوفارنکس (n= ۱۵۶)، سکوم (n= ۷۰) و کبد (n= ۷۰) حیوانات مذکور تهیه و کشت باکتریایی از آنها انجام گرفت. یافته ها نشان دادند که باکتری های زئونوز فوق الذکر در هیچ کدام از نمونه های آزمایش شده وجود ندارند ولی در ۷۸٪ از موش ها باکتری هایی که تحت شرایط معمول غیر بیماریزا هستند، یافت شدند که عمده آنها *Escherichia coli sp, Bacillus sp, Streptococcus sp* و *spp Entrobacter* بودند. نتایج حاصل از این مطالعه پیشنهاد می کند که موقع استفاده از این حیوانات در کارهای تحقیقاتی تهدیدی برای سلامت پرسنل و همچنین دخالت و میانکنش های باکتری های زئونوز فوق الذکر وجود ندارد. بهر حال در زمان استفاده از حیوانات آزمایشگاهی متعارف باید الزامات ایمنی کار با این حیوانات در نظر گرفته شوند.

واژه‌های کلیدی: زئونوزها، موش متعارف، آزمایش باکتریایی، کلنی پرورشی.

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