Vol.45, n. 3 : pp. 287-291, September 2002
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
A N I N T E R N A T I O N A L J O U R N A L

Health Status of Male Adult Wistar Rats from Two Experimental Animal Houses of UFMG: Leukocyte Counts, Feces and Lung Histological Exams

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ABSTRACT

A study was conducted to compare health status of male adult Wistar rats from two Experimental Animal Houses of UFMG with literature data of SPF (free from specific pathogens) and conventional rats. The animals were divided into two groups: Group I (n=10), rats from the experimental animal houses of FAFICH and Group II (n=10) from ICB and following aspects were studied: a) evident clinical signs (behavior modification, hair loss (alopecia), b) leukocyte counts, c) feces exam and d) histological study of the lungs. The rats did not show clinical signs. However, when compared with SPF and conventional rats, both the groups showed a significant increase ($p<0,05$) of leukocyte count. On feces exam we detected some parasites and on lung histological exam we observed fungus (Group I) and bacteria (Group II). These results showed that the health status of the rats was not satisfactory and required improvements in the conditions of the animal houses.

Keywords: Wistar rats, animal houses, cellular count

INTRODUCTION

The control of health status of any animal in an experiment is essential to avoid misunderstanding the results. This should be obtained by personal and environmental hygiene, control of environmental conditions such as ventilation, temperature, humidity, light, sounds, and routine microbiologic monitorization (Valero, 1990; Hardy, 1967).

Theories about environmental contaminati on and widespread diseases were established for the first time by Hypocrites (480-377 a.C) (Valero, 1990) but little improvement was done until the end of the 50's and the beginning of the 60's, when the concept of barriers were created (Hansen, 1994; Goldstein, 1978; Ganaway and Allen, 1986), in order to keep them free from disease. Since then, many animals classification have been used to separate in accordance with their sanitary quality and to guarantee the model quality. We used the same classification: i) conventional, animals without health control; ii) controlled, rats from an animal house with complex barrier system, able to define healthy colonies. Controlled animals are categorized in accordance with monitorization such as axenic, without microbia1 flora, gnotobiotics, defined flora, SPF (specific pathogen free), COBS (cesarean obtained barriers) and VAF (viral antibodies free) (Personal Communication *CEMIB-Unicamp*, 1998).

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The present study was undertaken to evaluate the health status of male adult Wistar rats from two experimental animal houses of UFMG, comparing it with literature data of SPF and conventional animals from animal houses of other research centers. All animals included in this study were from the animal house CEBIO - ICB (CEBIO - Centro de Bioterismo do Instituto de Ciências Biológicas - UFMG) and were kept for more then 6 months in two experimental animal houses of UFMG: FAFICH (Faculdade de Filosofia e Ciências Humanas) and ICB - (Instituto de Ciências Biológicas - C2 pavilion). In this study we used the following criteria as parameters: a) evident clinical signs (behavior modification, sneezing, coughing, hair loss or alopecia area, abnormal growth, diarrhea. b) leukocyte manual count, c) feces exam and d) histological study of the lungs.

MATERIALS AND METHODS

Animals

Twenty, male Wistar rats 7 month-old, apparently healthy, were kept in two independent animal houses located in different buildings. Animals from FAFICH, group I (n=10) were kept in individual cages since birth and animals from ICB, group II (n=10) were taken from different cages with at least 5 rats, then kept for two months in individual cage.

Sample collection and clinical signs

All animals were sacrified in the morning by decapitation and blood samples were imme diately collected from the cervical lesion in a syringe previously humidified with heparin (liquemine® 25000UI/ml). A skin and tooth exam was performed, followed by opening abdominal and thoracic cavities and resection of both lungs and the large and small intestines. At this time both cavities were checked for any possible macroscopic findings.

Clinical exam

Presence or absence of any clinical sign in the animal was fester, using the following criteria:

1. behavior modification (prostration, agitation, lethargy and aggressiveness);
2. skin lesions: hair loss, rarefied hair, areata alopecia and easy hair removal;
3. respiratory abnormalities (cough, sneeze, wheeze, hemoptysis);
4. diarrhea;
5. weight variation.

Differential count: mononuclear cells and granulocytes

Mononuclear cells and granulocytes were isolated by Ficoll-Hypaque gradient according to Bicalho et al. (1981), with slight modifications. The viability of each sample was higher than 95% as determined by the Typan Blue exclusion test. Blood sample (0.75ml) was added slowly and carefully on 0.75ml of *Ficoll-Hypaque* (density 1.12 and 1.08 respectively). All tubes were centrifuged at 100*g* (FANEN – 205 N) for 15 minutes, resulting in a mononuclear cells ring which was collected by Pasteur's pipettes and held in another tube. Plasma was selected and discharged.

The rest of the volume was centrifuged for the second time at 100*g* for 25 minutes in order to obtain granulocyte cells. The granulocyte ring was collected and transferred to another tube (as done with mononuclear cells). To each tube of both groups of cells 5ml of NaCl 0.9% was added and centrifuged for the third time at 50*g* for 30 minutes. The supernatant was discharged and the pellet re-suspended with NaCl 0.9% to a final volume of 1ml. Mononuclear cells and granulocytes were manually counted in an optical microscopic (x40) in Neubauer's camara. Results were expressed in cells/ml.

Parasitological exam

After sacrificing the animals, their bowels were taken out with its contents, conserved in formol 10% and observed in an optical microscope by direct exam.

Histological lung exam

Both lungs of each rat were taken and fixed in formol 10%, just after decapitation. The organ was shopped and processed by histotecnical Oma for 90 minutes and included in paraffin blocks. Slices of 5μm were taken from the blocks by microtome and fixed on slides. Slices of tissue were kept in a stove for 15 minutes and stained using Hematoxiline-Eosine, Gram, Gomory and Masson Staining (GMS) methods for each side of the lungs. All slides were examined on an optical microscope (x10, 40 and 100 fold) with Gram Stained slides observed under oil immersion.

RESULTS AND DISCUSSION

In both groups (I and II), no clinical abnormalities were found, i.e., they were assyntomatic, without any evidence of disease. The examination for parasites from animals of group I showed eggs and larva of *Syphacia obvellata* ranging from a discreet amount to severe infection. Eggs of *Aspiculuris tetraptera* were found in two animals. Histologically, the lungs showed an enlargement of alveolar spaces due to neutrophill-rich exsudant, associated to necrotic areas of supurative destruction of lung parenchyma, suggesting pneumonite and pleuritis. In these areas, GMS revealed fungus infection, made by multiple small grouped septated hyfas and some sporos. The other staining techniques did not show evidence of bacterial infection (Gram) or fibrosis (GMS). Due to technical limitations, the real etiology of the infection could not be clarified by the culture. However, we observed proportional increase of cell for granulocytes and mononuclear, higher compared to literature data (Ringler,1979; Hardy, 1967; Gordon, 1959), in a total of leukocytes of 11.8 x $10^3/\mu l$.

Stool test in animals of group II revealed eggs and larva of *Syphacia obvellata* less intense then group I. Eggs of *Hymenolepis sp* were present in 4 of the 10 animals from the group I. In the lung exam chronic bronchopneumonia in different stages of evolution were noticed, showing in some animals supurative intrabronchial neutrophilic exudant, clearly showing severe lesions, able to seriously affect the life and health status of these animals. In this group, GMS (for fungus) was negative. Hence, the etiology of this process could not be defined. The results are shown in Table 1:

Table 1 - Summary of clinical sign observation and laboratory evaluation of male adults Wistar rats from 2 units of UFMG (Groups I and II).

PARAMETERS	OBSERVATIONS	
	GROUP I	GROUP II
Clinical signs	None	None
Stool exam	*Syphacia obvellata* *Aspiculuris tetraptera*	*Syphacia obvellata* *Hymenolepis sp*
Lungs exam	Chronic Bronchopneumonia	Chronic Bronchopneumonia
Leukocyte count	Leukocytosis	Leukocytosis

Both groups showed evidence of infection. The result of leukocyte count is shown in Table 2. These findings were in agreement with Hansen, 1994 who also showed that the absence of clinical signs could not be interpreted as no infection. Leukocyte cells were increased in both groups (I and II). Comparing to Gordon's (Gordon, 1959) and Hardy's data (Hardy, 1967) for conventional male adult Wistar rats, our data showed a significant increase of leukocyte cells ($p<0.05$ Student "t" test), (approximately 78 and 93% for groups I and II, respectively). Comparing to SPF animals (Gordon, 1959) this increase was even higher (140 and 163%), confirming leukocytosis in animals from group I and II. However, this increase was not significant when compared to Coleman's data (Coleman et al., 1971) for conventional adult Wistar rats with mild pneumonia. The lungs histological results confirmed chronic pneumonia in animals, which could corroborate the evidence of increase in the leukocyte count. None of the bowel parasites found in these animals showed pulmonary cycle, so no direct relationship between both pathologies could be seen. However, any parasite could induce or transmit bacterial and viral disease, decrease or increase immunological response of the host (Farrar et al., 1986), which could possibly justify the association of both infections.

Our results showed that the health condition of the animals was not satisfactory and that could strongly interfere in experimental data obtained using these animals as models. One of the possibilities for this could be the present condition of the animal houses. Studies have shown that laboratory animals severely infected by *Syphacia obvellata* produced a lower biological product and were not reliable experimental models.

Table 2 - Comparison of numbers of leukocytes obtained from male adult Wistar rats from different animal house

		Granulocytes		Mononuclears		Leukocytes
Animal House	Type	$x10^3/\mu l$	%	$x10^3/\mu l$	%	$x10^3/\mu l$
FAFICH-UFMG (GROUP I)	Conv	3.21	27.12	8.6	72.88	11.81 ± 0,25
ICB- C2-UFMG (GROUP II)	Conv	1.74	13.18	11.2	86.82	12.94 ± 0,12
University of Notre Dame	SPF	0.73	14.86	4.0	81.46	4.91 ± 0,40
(Gordon,1959)	Conv	1.46	21.74	4.7	70	6.71 ± 0,23
Philadelphia (Hardy et al ,1967)	Conv	1.27	19	5.39	80.5	6.66 ± 0,52
Colgate-Palmolive Research Center (Coleman et al,1971)	Conv▲	1.60	14	9.83	86	11.44 ±0,36

Comparison done based on *t student* test.
▲ conventional animals with mild pneumonia.

This could make the experimental research cost higher (Ganaway et al., 1986). Animals with high severe parasital infection are not reliable for many scientific studies because the physiological responses and the cellular count are usually altered. In many conditions, a low parasital infection can be asymptomatic but under experimental conditions a lower animal resistance can provide unreliable or contradictory results, unable to be repeated (Farrar et al., 1986). The pathological effects of *Aspiculupis tetraptera* were the same as *S. obvelata* (Flynn, 1973). Infection by *Hymenolepis spp* can be latent or asymptomatic but, in some situations can occur constipation, catarrhal diarrhea, chronic weight loss and death can occur (Flynn, 1973).

The lung lesions found in both groups (I and II) seemed to be reversible. Thus, improving the maintenance and care of the animal houses is essential. Epidemiological measures such as sanitary control of the animal room, based on the decreasing number of animals per cage, a strict control of staff hygiene, control of temperature and ventilation in the animal room and specific treatment for the infectious agent are required. Heine, described several simple important hints for improving conditions of conventional animal houses.

In Campinas University (SP) there is a reference center of animal house monitorization and animal maintenance. At this center, conventional animal evaluation is made using several parameters such as microbiological cultures, soro logical exams for virus, bacteria and parasites, using indirect immunofluorescence (IIF), hemoagglutination inhibition, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) for several pathogens (Personal communication). All these methodologies are routinely used in CEMIB every six months.

Interestingly we have noticed that data about conventional animal houses solving problems and suggesting improvements are presently not published. It probably mean that the developed centers were already rid of these problems. In these centers the use of controlled animals are widespread and the aleatory factors that interfere in the experiment are lower.

Quantity data related in this work can confirm one of the most important problems that rese arches from UFMG are facing and could also be regarded by research sponsors as a new proposal of setting up and maintaining controlled animal houses in our university.

RESUMO

O controle do estado de saúde de cada animal que fará parte de um experimento é essencial para prevenir falsa interpretação dos resultados. Sendo assim, o objetivo do presente trabalho foi comparar as condições de saúde de ratos Wistar adultos, machos dos biotérios experimentais da FAFICH (n=10) e do ICB-Bloco C-2 (n=10) provenientes do mesmo biotério de criação (Centro de Bioterismo CEBIO-ICB) com dados da literatura de ratos SPF (livres de patógenos específicos). Foram utilizados para avaliação os seguintes critérios: a) sinais clínicos evidentes

(p. ex. alteração no comportamento, queda de pêlos), b) contagem manual de leucócitos, c) exame copro-parasitológico e d) necropsia de pulmão (exame anatomopatológico). Os animais não apresentaram sinais clínicos de doença. No entanto, quando comparados com ratos SPF, a contagem de leucócitos dos ratos provenientes dos dois biotérios foi significativamente diferente ($p<0,05$). Através dos exames parasitológicos e necropsia de pulmão foram detectados parasitas patogênicos e fungos presentes em processos inflamatórios. Nossos resultados indicam que as condições de saúde dos animais não são satisfatórias e uma das causas prováveis pode ser as atuais condições dos biotérios (talvez as condições do ambiente, alojamentos, população por gaiola, barreiras, divisão interna da área do biotério).

ACKNOWLEDGMENTS

We thank Dr. Ana Maria Guaraldo and Rovilson Gilioli (Unicamp), Dr. Ênio Cardillo Vieira (UFMG), Dalva Maria de Resende (Faculty of Medicine – UFMG), Maria Aparecida Resende (ICB – UFMG), Dr. Paulo Marcos Zeck (ICB – UFMG), Dr. Pérsio Godoy (Faculty of Medicine – UFMG), Dr. R. Hilton Girão (Faculty of Veterinary – UFMG), Carmencita Marcatti for making this work possible. Supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMIG (Fundação de Amparo à Pesquisa de Minas Gerais).

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Received: April 06, 2000;
Revised: February 13, 2001;
Accepted: December 07, 2001.