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BRIEF REPORT

Microbiological contaminations of laboratory mice and rats in conventional facilities in Argentina

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Abstract Routine microbiological monitoring of rodent colonies in animal facilities is essential to evaluate the health status of the animals used in research studies. In the present study, animals were examined for the presence of selected microbial infections. In order to determine the contamination rates of mice and rats in Argentina, animals from 102 conventional facilities were monitored from 2012 to 2016. The most frequent bacteria isolated were *Pseudomonas aeruginosa* and *Proteus* spp. The common parasites identified were *Syphacia* spp. and *Tritrichomonas* spp. Serological assays demonstrated the highest prevalence for Mouse hepatitis virus in mice and Sialodacryoadenitis virus in rats. The results indicate that there is a high incidence of infections, so it is suggested that an efficient management system and effective sanitary barriers should be implemented in conventional facilities in Argentina in order to improve sanitary standards.

PALABRAS CLAVE
Contaminaciones;
Instalaciones;
Ratones;
Ratas

Contaminaciones microbiológicas de ratones y ratas de laboratorio en bioterios convencionales de Argentina

Resumen Los controles microbiológicos de rutina en colonias de roedores en bioterios son esenciales para evaluar el estado de salud de los animales que se utilizan en las investigaciones. En el presente estudio se examinaron animales de bioterios de Argentina con el objeto de detectar la presencia de infecciones microbianas seleccionadas. Con el fin de determinar los porcentajes de contaminaciones en estos individuos, se controlaron animales de 102 bioterios convencionales entre 2012 y 2016. Las bacterias más frecuentes aisladas fueron *Pseudomonas aeruginosa* y *Proteus* spp. Los parásitos comunes identificados fueron *Syphacia* spp. y *Tritrichomonas* spp. Los ensayos serológicos demostraron la mayor prevalencia del virus de

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hepatitis del ratón en ratones y del virus de la *Syalodacryoadenitis* en ratas. Los resultados indican que hay una alta incidencia de infecciones, por lo que se sugiere que se debe implementar un sistema de gestión eficiente y barreras sanitarias eficaces en instalaciones convencionales en Argentina con el objeto de mejorar los estándares sanitarios.

Microbiological monitoring is the practice of scheduled and repetitive testing of an animal colony for evidence of selected microbial infections[3,4,8,9,11,12]. For many years, experiments failed because of the presence of diseases that killed animals during the assay or produced interferences. Therefore, it is important to determine the prevalence of infections in mice and rats colonies since this data will allow to know their sanitary condition[6,10]. In the case of Argentina, there is a lack of information and no data about this issue. International guidelines[6] recommend to perform the experiments with microbiological and genetically defined animals since Infections are considered a complicating factor in biomedical research. Establishing laboratory rat and mice health status is an important issue to be achieved worldwide since it is a requirement to comply with international standards regarding the animal quality[15]. The Experimental Animal Laboratory (LAE) at the Faculty of Veterinary Sciences-National University of La Plata (FCV-UNLP) is the former facility that has formally performed since 1996 microbiological monitoring for health surveillance of small rodents in Argentina. In the present study the microbiological status of 2972 mice and rats colonies from conventional facilities in Argentina was studied.

All the protocols used in this study have been approved by the Institutional Animal Care and Use Committee (IACUC) at the FCV-UNLP.

The animals were randomly taken according to age and sex as follows, 2 male and 2 females over 24 weeks old, 2 males and 2 females from 6 to 12 weeks old and 1 male and 1 female 3 weeks old. In the case of rats the samples were obtained from WKAH/Hok, WKY, LEW and F344 strains and SD stock and in mice BALB/c, C57BL/6 strains and Crl:CD1, CF1, NLAE: NIH (S)-Fox^{1nu} and NLAE: NIH (Swiss) stocks. The animals characteristics could be obtained in Charles River Laboratories (https://www.criver.com/), The Jackson Laboratory (https://www.jax.org/) or Taconic (https://www.taconic.com/)

The animals were euthanized by CO_2 gas in order to performed microbiological monitoring to survey the presence of the listed pathogens. It was considered the recommendation proposed by Federation of European Laboratory Animal Science Associations (FELASA) for the selection of the microorganisms to be monitored[10].

Blood collection was made by cardiac puncture. Blood was stored overnight at 4 °C in order to get the sera. Serum specimens were inactivated at 56 °C for 30 min and diluted in phosphate-buffered saline (PBS) (1:5) and stored at −20 °C until use[1].

The serological tests were carried out by using Indirect Immunofluorescence Assay (IFA)[5] for: Mouse hepatitis virus (MHV), Sendai virus (HVJ), Minute virus of mice (MVM), Theiler's mice encephalomyelitis virus (TMEV in rats, TMEV-GDVII in mice), Sialodacryoadenitis virus (SDAV), Kilham rat virus, *Mycoplasma* spp., Cilia-Associated Respiratory *Bacillus* (CAR *Bacillus*) and *Clostridium piliforme*. The IFA tests were performed by the method of Cherry et al., 1961[2]. Microagglutination test (MAT) was used to detect antibodies against *Corynebacterium kutscheri* and *Bordetella bronchiseptica*. It was carried out as described by Suzuki et al., 1986[13].

Samples for respiratory tract bacteriological monitoring were taken from the trachea by using a swab and from cecal content for digestive system bacteria. Samples were seeded on blood agar[a], McConkey agar[a], cetrimide agar[a] and FNC agar[a] and cultured as required. Culture tests were performed to isolate *B. bronchiseptica*, *Citrobacter rodentium*, *Corynebacterium kutscheri*, *Klebsiella* spp., *Mycoplasma* spp., *Pasteurella* spp., *Proteus* spp., *Pseudomonas aeruginosa*, *Salmonella* spp., *Staphylococcus aureus* and *Streptococcus pneumoniae*[5,10,14] according with microbiological culture methods.

Sources and manufacturers:

a: Britania S.A.

Simple biochemical tests were performed for the identification of the above bacteria according with Bergey's Manual of Systematic Bacteriology[8] and The Handbook of Laboratory Animal Bacteriology 2000[7]. In order to isolate *Mycoplasma* spp., the samples were cultured in PPLO broth[a], afterwards in PPLO agar[a] and observed 5–7 days after incubation to identify typical colonies.

Cellophane tape test, direct smears from duodenal and cecal contents and flotation method were performed in order to detect ectoparasites and endoparasites respectively[4].

In mice the results showed that the most frequent bacterium isolated was *P. aeruginosa* (49.59%); the second one was *Proteus* spp. (4.71%). At the serology, 35.19% of 1774 mice had antibodies against MHV. It was determined the presence of *Syphacia obvelata* and *Tritrichomonas* spp. in 34.26% and 25.84% respectively, in the monitored 1722 mice tested (Table 1).

P. aeruginosa and *Proteus* spp. were the most frequent bacteria isolated in rats with 66.7% and 4.36% respectively. The serology assay showed that 40.15% of the rats present SDAV. *Syphacia muris* was present in 38.83% and *Tritrichomonas* spp. in 35.15% of the rats examined (Table 2).

Table 1 Contaminations in laboratory mice in Argentina during 2012 to 2016 period.

Year	2012	2013	2014	2015	2016	Total	%
No. of mice tested for bacteriology	430	340	218	348	382	1718	
Citrobacter rodentium	0	0	0	0	0	0	
Corynebacterium kutscheri	0	0	0	0	0	0	
Klebsiella oxytoca	0	0	0	0	0	0	
Mycoplasma spp.	1	0	0	0	0	1	0.06
Pasteurella pneumotropica	0	0	0	0	0	0	
Proteus spp.	27	41	7	6		81	4.71
Pseudomonas aeruginosa	213	207	99	170	163	852	49.59
Salmonella spp.	0	0	0	0	0	0	
Staphylococcus aureus	0	0	0	0	0	0	
Streptococcus pneumoniae	0	0	0	0	0	0	
No. of mice tested by serology	416	327	289	339	403	1774	
CAR *bacillus*	0	7	2	0		9	0.51
Clostridium piliforme	21	25	0	8	134	188	10.60
Corynebacterium kutscheri	37	30	63	23	77	230	12.97
Mycoplasma pulmonis	72	54	28	30	108	292	16.46
MHV	134	159	130	109	91	623	35.12
HVJ	32	54	39	28	59	212	11.95
MVM	32	65	70	35	65	267	15.05
TMEV-GDVII	0	84	77	33	72	266	14.99
No. of mice tested for parasitology	430	344	218	348	382	1722	
Entamoeba muris	6	16	9	0	3	34	1.97
Eimeria spp.	5	12	0	1	0	18	1.05
Giardia muris	17	16	7	11	4	55	3.19
Spironucleus muris	38	16	14	11	7	86	4.99
Tritrichomona spp.	95	60	59	90	141	445	25.84
Syphacia obvelata	154	170	96	97	73	590	34.26
Aspiculuris tetraptera	5	2	4	4	2	17	0.99
Hymenolepis nana	3	3	1	2	0	9	0.52
Myobia musculi	42	6	10	1	0	59	3.43
Myocoptes musculinus	36	24	31	24	6	121	7.03
Poliplax spinulosa	0	0	0	0	0	0	
Sarcoptes scabiei	0	0	0	0	0	0	

This study reports on the microbiological status of laboratory mice and rats housed in 102 conventional facilities in Argentina from 2012 to 2016. One of the major contaminations identified was *P. aeruginosa*. It was present in 852 mice out of 1718 (49.59%) and 673 rats out of 1009 (66.70%). Although this bacteria is considered an opportunistic pathogen in immunocompetent animals, it produces interferences in the research results when immunodeficient mice are used. In this animal model *P. aeruginosa* causes severe infections and aggravates this disease resulting in significant morbidity and mortality. The major viral contaminations were MHV in mice facilities; it is considered a fatal pathogen. We have found antibodies against MHV in 623 animals out of 1774 (35.19%). Among fatal pathogens of rats SDAV was present in the colonies in a 40.15%.

Nonpathogenic protozoa were detected in facilities of both mice and rats. The most common were *Tritrichomonas* spp. and the pinworms *S. obvelata* and *S. muris*.

Contamination rates of some tested microorganisms (*P. aeruginosa*, *Proteus* spp., *M. pulmonis*, *MHV*, *Giardia* spp., *Tritrichomonas* spp., *Myocoptes musculinus* in mice and *P. aeruginosa*, *Proteus* spp., *Mycoplasma* spp., *Tritrichomonas* spp., *S. muris*, *Spironucleus muris* in rats) have increased because the number of mice and rats used in biomedical research in Argentina has increased in the past 15 years.

All the isolated microorganisms have been identified in facilities worldwide, they are common mice and rats pathogens, hence it can be confirmed that the contaminations detected in Argentina do not differ from those in any part of the world.

We also have to consider that in Argentina there are no regulations regarding the care and use of experimental animals and thus no requirements; this fact probably contributes with the poor microbiological quality that have been found in mice and rats colonies. Therefore, it would be important that the facilities in Argentina perform health monitoring programs in order to decrease pathogen contaminations.

In conclusion, the Argentine scientific community should be aware of the role of rats and mice subclinical infectious and of the need to improve laboratory animals

Table 2 Contaminations in laboratory rats in Argentina during 2012 to 2016 period.

Year	2012	2013	2014	2015	2016	Total	%
No. of rats tested for bacteriology	244	197	110	195	263	1009	
Bordetella bronchiseptica		0	0	0	0	0	
Citrobacter rodentium		0	0	0	0	0	
Corynebacterium kutscheri		0	0	0	1	1	
Klebsiella oxytoca		0	0	0	0	0	
Mycoplasma spp.	1	0	0	0	0	1	0.10
Pasteurella pneumotropica		0	0	0	0	0	
Proteus spp.	10	28	5	1	0	44	4.36
Pseudomonas aeruginosa	140	149	82	130	172	673	66.70
Salmonella spp.		0	0	0	0	0	
Staphylococcus aureus		0	0	0	0	0	
Streptococcus pneumoniae		0	0	0	0	0	
No. of rats tested by serology	259	211	198	195	335	1198	
Bordetella bronchiseptica	35	45	68	30	38	216	18.03
CAR *bacillus*		30	44	3	9	86	7.18
Clostridium piliforme	21	40	2	0	87	150	12.52
Corynebacterium kutscheri	37	53	62	20	86	258	21.54
Mycoplasma pulmonis	122	89	64	36	78	389	32.47
SDAV	141	97	98	47	98	481	40.15
Kilham rat virus	120	41	10	8	13	192	16.03
HVJ	42	30	27	6	29	134	11.19
MVM		32	12	5	4	53	4.42
TMEV		40	19	8	19	86	7.18
No. of rats tested for parasitology	255	197	110	195	273	1030	
Entamoeba muris	0	1	0	2	9	12	1.17
Eimeria spp.	14	0	0	8	0	22	2.14
Giardia muris	35	13	0	0	13	61	5.92
Spironucleus muris	61	34	20	17	80	212	20.58
Tritrichomona spp.	89	71	39	48	115	362	35.15
Syphacia muris	116	94	55	49	87	401	38.93
Aspiculuris tetraptera	6	10	3	1	0	20	1.94
Hymenolepis nana	3	9	0	0	4	16	1.55
Myobia musculi	16	2	0	0	0	18	1.75
Myocoptes musculinus	3	0	4	0	2	9	0.87
Poliplax spinulosa	2	0	0	0	3	5	0.49
Sarcoptes scabiei	0	0	0	0	0	0	

microbiological status by establishing SPF barrier facilities and regular health monitoring programs in order to work in compliance with international standards.

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Authors' contributions

All authors contributed to the conception, analysis, or interpretation of data; drafted the manuscript; critically revised the manuscript; and gave final approval.

Conflict of interest

The authors declare that they have no conflicts of interest.

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