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The bacteriological status of a specific-pathogen-free animal production building and of its staff and the microbiological integrity of the animals one year after the building was commissioned

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SUMMARY

Bacteriological contamination of a specific-pathogen-free (SPF) animal production building was more extensive after one year of operation than at the time of commissioning. Throat and nose swabs taken from the staff yielded a number of undesirable organisms, in particular, on one occasion, a Group A streptococcus. It is recommended that a closer bacteriological scrutiny of the staff of SPF production buildings ought perhaps to be made in the light of these findings.

INTRODUCTION

The bacteriological status of a new SPF animal production building was assessed before and after fumigation (Taylor, 1974). During the subsequent twelve months large numbers of animals have been produced, some 4550 entries through the barrier have been made and various quantities of maintenance materials have been introduced.

This paper records the results of an assessment after twelve months of the microbiological status of the animals and of the bacteriological status of the building and staff.

MATERIALS AND METHODS

Ninety-five sites in the building were examined by the method described by Taylor (1974). The cultures obtained after 72 hr. incubation at 37° C. were subcultured to 5 % horse-blood agar plates and incubated for a further 36 hr. The organisms thereby isolated were identified according to their morphology and reaction to routine laboratory tests.

Animals

The animals in the building were examined each month for the presence of proscribed microflora for animals of **** rating, according to the methods recommended by the MRC Laboratory Animals Centre, Accreditation Microbiological Advisory Committee (MRC/LAC, AMAC). Briefly, this publication recommends methods of isolation and examination for proscribed microflora and indicates the preferred sites of culture. Pathogenic or potentially pathogenic organisms are catalogued in another MRC publication (MRC, 1972).

Table 1. *Results of bacteriological examination of various sites in a S.P.F. animal product building one year after it had been commissioned*

		Sites in each area		Aerobic spore-bearers	*Ps. aeruginosa*	*Proteus* spp.	*E. coli*	*Aerobacter* spp.	Strep. viridans	Strep. group D	agulase neg. taph.	Diph-theroids
		No.	Pos.									
Anima	ıs (10)	50	42	8	4	1	5	3	5	1	13	1
Service		10	6	2	2	—	1	1	1	1	—	—
Office		5	3	—	—	—	1	—	1	—	1	—
Mess		5	5	1	3	—	—	1	—	—	3	1
Store		5	2	—	1	—	—	—	—	—	2	—
Toilets												
Men	ide)	5	4	—	—	—	—	—	1	—	4	—
Men	tside)	5	4	2	1	—	—	—	—	—	1	—
Ladi	ıside)	5	3	—	—	—	—	—	—	—	3	—
Ladi	utside)	5	5	2	1	—	1	1	1	—	1	—
T		95	74	15	12	1	8	6	9	2	28	2

Table 2. *Results of the microbiological and parasitological examination of random samples of animals produced in a S.P.F. animal production building up to one year after it was commissioned*

	Mice	Rats	Guinea-pigs	Total
Staphylococci				
Coagulase-positive	1 (2)	0	0	1 (1)
Coagulase-negative	5 (10)	1 (5)	1 (13)	7 (9)
Streptococci				
Viridans	37 (77)	16 (80)	8 (100)	61 (80)
Group C	0	1 (5)	0	1 (1)
Group D	0	1 (5)	0	1 (1)
Haemophilus influenzae	0	4 (20)	0	4 (5)
Proteus spp.	23 (48)	17 (85)	1 (13)	41 (54)
Escherichia coli	23 (48)	16 (80)	8 (100)	47 (62)
Diphtheroid bacilli	3 (6)	0	2 (25)	5 (7)
Lactobacilli	44 (92)	8 (40)	2 (25)	54 (71)
Mycoplasmas	0	0	0	0
Ecto-/endo-parasites	0	0	0	0
Significant serum titres against				
Sendai virus	0	0	0	0
Leptospiras*	0	0	0	0
Total animals tested	48	20	8	76

Figures in parentheses are percentages.
* Serum samples tested against 16 different antigens.

Staff

At the time the animals were monitored, throat and nose swabs from members of the staff servicing the unit were spread on 5 % horse-blood agar plates and incubated for up to 36 hr. at 37° C. Organisms recovered were identified according to accepted bacteriological principles.

RESULTS

The building

Organisms were isolated from 74/95 (78 %) of sites examined (Table 1). This compares with 13/100 (13 %) of sites examined when the building was brought into commission after fumigation (Taylor, 1974). The types of organisms recovered were similar to those present at the time of the first examination. Comparison of these two examinations shows that there was a 13 % increase in the number of sites contaminated with Gram-negative flora (3/13 23 % 1973:27/74 36 % 1974) and an 11 % decrease in the number of sites contaminated with Gram-positive cocci (staphylococci and streptococci) (8/13 64 %:39/74 53 %) during the year. The percentage of aerobic spore-bearing organisms remained constant and only a few diphtheroids were identified.

The animals

The results of tests on 76 animals are given in Table 2. The background flora was found to be fairly constant in all species, the only variation being in the rats from which groups C and D streptococci and *Haemophilus* species were isolated.

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Table 3. *Results of the bacteriological examination of nose and throat swabs from staff servi the S.P.F. animal production building*

Orga	s isolated	A* N	A* T†	B N	B T	C N	C T	D N	D T	E N	E T	F N	F T	G N	G T	H N	H T	I N	I T	Totals N	Totals T
Staphy	ci																				
Coag	positive	2	—	1	—	5	2	—	—	1	—	2	1	—	1	—	—	—	—	11	4
Coag	negative	4	—	3	1	2	1	2	—	3	—	1	—	1	—	—	—	1	—	17	2
Strept																					
Viric		2	6	—	7	1	10	1	5	—	4	1	1	2	2	1	1	—	1	8	37
Grou		—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1
Grou		—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Not	able	—	3	—	1	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	6
Neisser	*arrhalis*	—	—	—	2	—	1	—	1	—	1	—	—	—	—	—	—	—	—	—	5
Haemo	*influenzae*	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—
Lacobs		—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Proteus		3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—
E. coli		—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	—
Diphth	s	1	1	1	2	—	1	—	—	—	1	1	—	—	—	—	—	—	—	3	5
Staphy	*us albus*	—	—	2	3	2	3	2	1	—	—	—	—	1	2	1	—	—	—	8	9

* St ference: A–F = permanent staff; G–I = occasional staff. At no one time during the period of the observatio re all nine members of the s available for swabbing at the same time, owing to absence through illness or other cause.

† N; ; T, throat.

‡ Pr ed organism (grouped by Fuller's method).

The group C streptococci and the *Haemophilus* may have been of human origin (see Table 3). There was no evidence of ecto- or endo-parasites nor of *Mycoplasma* species, and significant antibody titres to Sendai virus or leptospira were not demonstrated. Screening of mice for persistent tolerant infection (PTI) to lymphocytic choriomeningitis (LCM) virus in mice showed that all of 38 mice tested were susceptible to challenge with the Pirbright LCM strain and no immunity was demonstrated. The colony from which these mice were derived may therefore be considered to be LCM-free.

The staff

Eleven of 46 nose swabs and 4/46 throat swabs of staff examined on ten occasions yielded coagulase positive staphylococci (Table 3); *Haemophilus influenzae* was isolated from the noses of two persons; Group A streptococci (proscribed for **** animals) were isolated from the throat of another member of staff, as also was a Group C streptococcus; *Neisseria catarrhalis* was isolated from throat swabs on five occasions. With the exception of the Group C streptococcus and haemophilus, none of the fore-mentioned organisms was recovered from the animals.

DISCUSSION

During the first year of operation of the SPF barrier system for producing mice, rats and guinea-pigs at this Institute, no proscribed organisms were isolated from the animals or from various sites within the building. However, a member of the staff contracted a throat infection associated with Group A streptococci; spread of this organism to the animals was avoided by the person reporting the condition immediately and remaining outside the barrier until it had abated.

A change in the bacteriological status of the building compared with that of the year previously, was of a minor nature and consisted of an alteration in the degree of contamination rather than the occurrence of new species. This was evidenced by the increase of the Gram-negative coliform types and the decrease in the Gram-positive cocci. It is possible that this situation may be attributed to two causes: (1) the increase in the number of animals, and therefore of the faecal contamination of the environment that their routine husbandry creates and (2) the regular and systematic use of a surface acting ampholytic biocide as a cleaning/disinfecting agent. Unpublished work in the Disinfection Reference Laboratory, Colindale, has shown it to be comparatively ineffective against Gram-negative flora although more active against Gram-positive organisms. These agents are more seriously inactivated by hard water and by organic material than are most other disinfectants. They are also severely inactivated by wood, cork, rubber, cotton, nylon and certain plastics (J. C. Kelsey & I. M. Maurer, personal communication). Those responsible for bacteriological tests of surfaces which have been treated with ampholytic disinfectants may be reminded that these agents are highly bacteriostatic. They must be neutralized to enable surviving organisms to grow and make their presence known (MacKinnon, 1974).

The appearance in the rats of *Haemophilus* species and Group C streptococci is

of particular interest because, although not proscribed organisms for **** animals, they have not hitherto been observed in our SPF animals. Both of these organisms were isolated from two members of staff about two months before their appearance in the rats, suggesting infection from a human source. If this were the case it demonstrates the ease with which cross-infection occurs and emphasizes the extreme care which is required in monitoring staff for proscribed organisms and in selecting staff of high integrity, who can be relied upon to bring apparently minor cases of illness to the attention of their supervisor, before entry to the unit is contemplated. *Neisseria catarrhalis* was not observed in any of the animals although it was isolated from the staff on five occasions.

The recovery of proscribed micro-organisms from a member of the staff indicates the need for frequent bacteriological examination of nose and throat swabs (weekly, perhaps, instead of monthly). There would also appear to be a strong case for conducting a microbiological survey of the building such as that described in this paper, at least annually. The work involved is little compared with the tremendous cost if a 'breakdown' should occur.

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