Scand. J. Lab. Anim. Sci. No. 2 . 1990 . Vol. 17

An improved technique for the decontamination of barrier units contaminated with Bacillus piliformis strains of rat origin

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INTRODUCTION

A majority of European rat colonies today seems to be infected with *Bacillus piliformis* (*Kraft & Meyer* 1990). The infection is mostly observed only by a high prevalence (50–80 %) within the colony of rats with antibodies against this microorganism, while clinical symptoms known as Tyzzer's disease are seldom and mostly related to certain strains of inbred rats (*Hansen et al.* 1990). Also stress tests using predinisolone results in a higher incidence of positive results within certain inbred strains of rats (*Hansen et al.* 1990). The antibodies can be eliminated by traditional rederivation techniques (*Hansen et al.* 1989). However, transferring of seronegative rats from isolator to a barrier unit formerly used for seropositive rats results in seroconversion within a very short period (*Illgen & Kouchakji* 1989).

The usual technique for decontamination of animal units includes the use of aldehydes as active desinfectant, e. g. formalin or glutaraldehyde. *Ganaway* (1980) showed that a 30 minutes exposure of spores of *B. piliformis* to an aqueous solution of formaldehyde only resulted in an effectiveness of 88 % spore killing, while 5 minutes exposure to peracetic acid or sodium hypochlorite resulted in 100 % inactivation of the spore suspension. Also heat-treatment at 80 centigrade for 30 minutes was shown to be 100 % effective.

At our laboratory glutaraldehyde desinfection of a unit followed by the introduction of gnotobiotic rats seronegative to *B. piliformis* results in the seroconversion within three weeks.

The aim of this study has been to develop a method depending on heat and peracetic acid as active desinfectants when desinfecting barrier units previously used for *B. piliformis* seropositive rats.

MATERIALS AND METHODS

Barrier unit

A barrier unit of 26 square metres floor area and 280 cm from floor to ceiling was used for the study. Entrance to the unit for staff was through a three room shower lock. Entrance to the unit for materials was through a desinfection lock. The unit was filled with autoclaved cages, bedding and diets before desinfection of the room. No new materials were introduced into the unit during the periods described in table 1. Ventilation was equipped with absolute filtres for the incoming air. Air was changed 10–12 times/hour. Temperature was kept at 22 centigrade ± 2. The light cycle was 6,00–18,00.

Desinfection with glutaraldehyde

1. Washing with water containing soap and phosphoric acid (Codaxid, Codan Chemicals, DK-2880 Bagsværd).
2. High pressure cleaning with a 0.5 % solution of NaOH.
3. High pressure cleaning with water without desinfectants.
4. Heating with gas canon (Tropic Warmluftgebläse, Infra-kolb, D-8510 Fürth) for 3 hours, temperature reaching 65 centigrade.
5. Desinfection with 20 % glutaraldehyde (Glucid, Superfos Biosector, DK-2950

Vedbæk) in aerosol using 1 litre of Glucid per 280 m^3.

Desinfection with peracetic acid

1. Washing with water containing soap and phosphoric acid (Codaxid, Codan Chemicals, DK-2880 Bagsværd).
2. High pressure cleaning with a 0.5 % solution of NaOH.
3. High pressure cleaning with water without desinfectants.
4. Heating with gas canon (Tropic Warmluftgebläse, Infra-kolb, D-8510 Fürth) for 8 hours every day for three days, temperature reaching 80 centigrade.
5. Desinfection with glutaraldehyde (Glucid, Superfos Biosector, DK-2950 Vedbæk) in aerosol using 1 litre of Glucid per 280 m^3.
6. Desinfection by manually spraying a 2 % solution of peractic acid (Divosan Forte, Diversey, DK-2730 Herlev) until everything within the room was wet with the solution.

Test for antibodies to B. piliformis

Test for antibodies was performed by dr. V. Kraft, Central Institute of Laboratory Animal Breeding, Hannover, FRG, using immunofluorescent assay (IFA). A titre of 1:20 or above was considered as positive.

Introduction of rats

Until October 1987 the barrier unit was used for the maintenance of a seropositive Lew/Mol rat colony. These rats were removed in October 1987 and the room was desinfected by the glutaraldehyde method described above. A colony of 10 male and 10 female germ-free adult SHR/Mol rats with litters was transferred from an isolator via a wall cylindre to the unit. The wall cylindre was cleaned using 2 % peracetic acid. Three weeks after 5 males were removed for serologic test for antibodies.

Hereafter the unit was used for breeding of different inbred rat colonies all seropositive to *B. piliformis*. One of these colonies was a BB/Wor/Mol-BB colony with rats showing clinical symptoms of Tyzzer's disease.

In October 1989 the unit was emptied and desinfected by the glutaraldehyde method. 10 male and 10 female adult SHR/Mol rats with litters, 10 male and 10 female adult BB/Wor/Mol-BB rats with litters, and 3 male and 3 female BUF/Mol were introduced from germ-free isolators as described above. The rats were then bred within the room. Nine weeks after the introduction 5 adult male rats were sampled for serologic testing. 16 weeks after introduction 10 adult male rats were sampled for serologic testing.

RESULTS

Results of serologic testing in historical order are given in table 1.

Table 1. Results of serologic testing using immunofluorescent assay (IFA) for antibodies to *B. piliformis* in rats introduced into a barrier unit desinfected with two different types of desinfectants: Glutaraldehyde and peracetic acid.

Week no		
Study of glutaraldehyde desinfection		
0 (Oct 87)	Glutaraldehyde desinfection and introduction of seronegative SHR-Mol rats.	
3	IFA (No tested/No positive):	5/3
Study of paracetic acid desinfection		
0 (Oct 89)	Peracetic acid desinfection and introduction of seronegative SHR/Mol, BB/Wor/Mol-BB and BUF/Mol rats	
9	IFA (No tested/No positive):	5/0
16	IFA (No tested/No positive):	10/0

DISCUSSION

The observation of seroconversion within three weeks after introduction of seronegative rats into the glutaraldehyde desinfected units is an accordance with observations normally done at our and other breeding centres. If the units have not previously been used for rats, seroconversion can be avoided (*Hansen et al.* 1989, *Illgen & Kouchakji* 1989). This indicates that the microorganisms is not eliminated during the normal desinfection procedures using e. g. aldehydes. The use of peracetic acid and heat in combination with the use of aldehyde desinfection seems to be more efficient in decontaminating the unit.

The results of this study indicate that the antibody titre observed in IFA is connected with the presence of a certain microorganism, but not that this microorganism is *B. piliformis*. However, these observations in vivo are in accordance with the in vitro investigations of *B. piliformis* (*Ganaway* 1980).

Three groups of resistant microorganisms are possible survivors of a decontamination procedure of a rat unit: Parvoviruses, helminth ovae and spore-forming organisms like *B. piliformis*. One will have to consider the need for a specific desinfectant against each group as in the above described procedure: Aldehydes against parvoviruses, NaOH against helminth ovae and peracetic acid against spores.

Summary

Desinfection of animal units is normally based on the use of aldehydes as desinfectants. This article describes a technique for desinfection of animal units in which the use of glutaraldehyde is supplemented with the use of heat and peracetic acid. This method is, in contrast to the use of aldehydes alone, effective against spores of *Bacillus piliformis* of rat origin, which is in accordance with in vitro investigations.

Sammendrag

Desinfektion af dyrerum bygger normalt på anvendelsen af aldehyder som desinfektionsmiddel. Denne artikel beskriver en teknik til desinfektion af dyrerum, der supplerer anvendelsen af aldehyder med anvendelsen af varme og pereddikesyre. Denne metode er i modsætning til metoder, der udelukkende bygger på anvendelse af aldehyder, effektiv imod sporer af *Bacillus piliformis* fra rotter, hvilket er i overensstemmelse med in vitro forsøg.

Yhteenveto / K. Pelkonen

Koe-eläinyksiköiden desinfiointi suoritetaan tavallisesti käyttäen aldehydejä. Tässä artikelissa kuvataan menetelmä jossa käytetään yhdessä prertikkahappoa ja glutaraldehydiä. Menetelmäon tehokas myös rotar *Bacillus piliformis* – spoorium tuhoamisessa.

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