

# Comment and answer to the paper entitled Probiotic Biotherapy for Eradication of a Potential Pathogen in a Commercial Rat Breeding Colony

Comment by **Ron Boot\***

Section of Laboratory Animal Microbiology, Laboratory for Infectious Diseases,  
National Institute of Public Health and the Environment, Bilthoven, The Netherlands

With interest I read the paper by Collinder E, Bergsted J, Persson A-K, Norin E and Midtvedt T: Probiotic biotherapy for eradication of a potential pathogen in a commercial rat breeding colony. *Scand. J. Lab. Animal Sci.* 2008, 35, 199-201.

The paper describes a study in which *Lactobacillus reuteri* was dosed to rats to clear them from (unspecified) Lancefield group G  $\beta$ -haemolytic streptococci that were detected in vaginal samples.

I would like to raise two points.

## **1. Was the study necessary?**

The study was undertaken 'as these microbes are on 'black list of FELASA''. Reference is made to the recommendations for the health monitoring of rodent and rabbit colonies (Nicklas *et al.*, 2002). The authors state that 'efforts had to be undertaken to eliminate them [i.e. the streptococci] from the animals'.

The FELASA recommendations suggest to monitor SPF colonies of rodents and rabbit for various infectious agents that might impact animal based research. The recommendations however do not (and can not) require that animals used in research must be free from the agents listed.

Group G streptococci comprise *S. dysgalactiae* subspecies *equisimilis* and *S. canis* (Kilian, 2005). Both species have not been reported in natural infections in rats. Although Kohn & Clifford (2002) state that ' $\beta$ -haemolytic streptococci are present in

many rats', there is only one report on clinical infection in rats by unspecified group C streptococci (Ahern *et al.*, 1979). Collinder *et al.* do not report clinical signs in their rats nor any effect on the outcome of animal experiments by the streptococci. Animal caretakers are likely the main source of bacteria introduced into SPF colonies. Bacterial flora of SPF-animals show a qualitative and quantitative evolution (Perrot, 1976; Salzman *et al.*, 2002). Some of the introduced bacteria are only temporarily present and are replaced by other species. The  $\beta$ -haemolytic streptococci might have disappeared spontaneously from the colony.

## **2. Was the study properly done?**

The authors decided to use a probiotic bacterial strain as a 'biotherapeutic agent', namely a rat strain of *Lactobacillus reuteri* to eliminate the  $\beta$ -haemolytic streptococci.

There has been an attempt to prevent the introduction of *Staphylococcus aureus* into a newly established colony of Han:NMRI-nu mice by preassociation with the rodent-specific *Staphylococcus sciuri*. Despite the successful colonization of the mice with *S. sciuri* the establishment of *S. aureus* into the colony was not impeded (Wullenweber *et al.*, 1989). The bioexclusion principle has been successfully used by the deliberate dosing of complex

\*Correspondence: R. Boot

Section of Laboratory Animal Microbiology, Laboratory for Infectious Diseases, National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

Tel +31 30 274 3432

Fax +31 30 274 4418

E-mail r.boot@rivm.nl

flora such as the so-called colonization resistant enteric flora (*Van der Waaij et al., 1971*), which can protect rodents and rabbits against colonization by gram negative opportunistic infection (*Boot et al., 1985, 1989*).

There were 3000 females and 100 males in the rat colony. All rats were treated 4 times by dosing *L. reuteri* via the drinking water and swabbing the vagina or penis of each individual animal, using a new swab per animal (why?).

The authors did not attempt to culture *L. reuteri* from the rats. It remains therefore unclear if the bacterium would have been able to colonize the genital mucosa after a single treatment. As the rats were used as breeding animals colonization of the mucosa of the penis would have been sufficient to distribute the bacterium to the females. That would have saved a lot of work and 12.000 swabs.

After administration of the *L. reuteri* no  $\beta$ -haemolytic streptococci were detected in 20 randomly taken samples, presumably from the females.

In the rat vagina viable counts for streptococci were found 1000 times higher in estrus than in anestrus (*Larsen et al., 1976; Noguchi et al., 2003*).

The rat estrus lasts about 14 hrs which is 25-30% of the duration of the cycle. This implies that of the 20 rats examined 4-7 only were likely in estrus and that the majority of the rats were in a phase in which viable counts of streptococci are expected to be low.

In the study described here *L. reuteri* was dosed to all rats. Unfortunately the authors did not include a control group of untreated rats, so it cannot be excluded that the 'problem' would have been resolved without any treatment.

I agree with the authors that 'use of probiotics as biotherapeutic agents should be considered when undesirable bacteria are found in laboratory animals', as the bioexclusion approach might be an alternative to eradication and rederivation.

I have doubts however about the necessity of the study, the way it was carried out and I feel that the title of the paper suggests too strongly that the 'biotherapy' was a success.

## References

- Ahern PF, RK Archer & S Sparrow*: An infection in rats caused by  $\beta$ -haemolytic streptococci of group C. *Vet. record* 1979, 104, 507-508.
- Boot R, JP Koopman, BC Kruijt, RM Lammers, HM Kennis, A Lankhorst, JWMA Mullink, AM Stadhouders, H de Boer, GW Welling & MPC Hectors*: The 'normalization' of germ-free rabbits with host-specific caecal microflora. *Lab. Animals*, 1985, 19, 344-352.
- Boot R, JP Koopman, Kruijt BC, RMLammers, HM Kennis A Lankhorst, GW Welling & MPC Hectors*: The 'normalization' of germ-free guinea pigs with host-specific caecal microflora. *Lab. Animals*, 1989, 23, 48-52.
- Kilian M*: Streptococcus and Lactobacillus. Ch. 3 in: Topley and Wilson's Microbiology and Microbial Infections, vol. 2 10<sup>th</sup> ed. (Boriello SP, PR Murray, G Funke eds.) Hodder Arnold, London 2005.
- Kohn DF, CB Clifford*: Biology and Diseases of rats. Ch. 4 in: Fox JG, L Anderson, FM Loew, F Quimby eds.) *Laboratory Animal Medicine* 2<sup>nd</sup> ed. Academic Press, New York 2002.
- Larsen B, AJ Markovetz & RP Galask*: Quantitative alterations in the genital microflora of female rats in relation to the estrous cycle. *J. Infect. Dis.*, 1976, 134, 486-489.
- Nicklas W, P Baneux, R Boot, T Decelle, AA Deeny, B Illgen-Wilke & Fumanelli M*: Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. *Lab. Animals*, 2002, 36, 20 – 42.
- Noguchi K, K Tsukumi & T Urano*: Qualitative and quantitative differences in normal vaginal flora of conventionally reared mice, rats, hamsters, rabbits, and dogs. *Comp. Medicine*, 2003, 53, 404-412.
- Perrot A*: Evolution of the digestive microflora in a unit of specified pathogen free mice: efficiency of the barrier. *Lab. Animals*, 1976, 10, 143-156.
- Salzman NH, H de Jong, Y Paterson, HJM Harmssen, GW Welling & NA Bos*: Analysis of 16S

libraries of mouse gastrointestinal microflora reveals a large new group of mouse intestinal bacteria. *Microbiol.*, 2002, 148, 3651-3660.

Van der Waaij D, JM Berghuis- de Vries & JEC Lekkerkerker-van der Wees: Colonization resistance of the digestive tract in conventional and antibiotic treated mice. *J.Hyg. (Cambridge)*,

1971, 69, 405-411.

Wullenweber-Schmidt M, W Lenz & K Brön-nemann: Can the association of athymic mice (Han:NMRI-nu) with *Staphylococcus sciuri* prevent infection with *Staphylococcus aureus*? Experiences from a field study. *Zeitschr. Versuchstierk.*, 1989, 32, 49-56.

Answer by *Eje Collinder<sup>1,2,\*</sup>, Tore Midtvedt<sup>1</sup> & Elisabeth Norin<sup>1</sup>*

<sup>1</sup>Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

<sup>2</sup>Mälaren Horse Hospital, Sigtuna, Sweden

Thank you for allowing us to answer questions raised by Dr R Boot.

**Question 1: Was the study necessary?**

Yes, The situation was as follows. When animals are found to harbour microbes that are on the FELASA list, most of the laboratory animal veterinarians in Sweden do not recommend their customers to buy such animals. We are aware of the fact that streptococci group G can be found in many animal species, both wild and domestic, without causing any disease. The animals at the animal breeding facility looked healthy but nobody wanted to buy them.

**Question 2: Was the study properly done?**

- Our reason for choosing *Lactobacillus reuteri* was very simple. The strain had been isolated from a healthy rat. The principle using lactobacilli for treatment of vaginosis is well established in human medicine.

- One swab per animal: we did not want to transfer any microbial agent from animal to animal. This is a well-established principle in human medicine.
- Control animals: we should have liked to have controls but the mere fact is that this study was performed in a commercial breeding farm partly excluded that possibility. It goes without saying that we could not take a any risk of transferring any pathogen organisms around in the farm.

Hopefully, after reading our article and these comments, other researchers will have the opportunity to further evaluate the strategy of eradicating tentative pathogens in a biotherapeutic way.

We should also like to draw FELASA's attention to their list. However, that topic is further commented upon in a recent submitted report, entitled *Intestinal microflora functions in laboratory mice claimed to harbor a "normal" intestinal microflora. Is the SPF concept running out of date?* by E Norin and T Midtvedt.

\*Correspondence: Eje Collinder  
S. Rudbecksg. 16, SE-752 36 Uppsala, Sweden  
Tel. +4618536531, +46733925711  
Fax +46859254032  
E-mail eje.collinder@privat.utfors.se