

***A Pasteurella pneumotropica* Strain of Mouse Origin Colonizes Rats but is Out Competed by a *P. pneumotropica* Strain of Rat Origin**

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Summary

Mouse and rat *Pasteurellaceae* have been found to differ in phenotypic characteristics but both animal species may be infected by each other's bacterial strains. We explored the possibility that a mouse *Pasteurella* strain colonizing rats would be out competed by a subsequent infection of a rat *Pasteurella* strain.

Rats were dosed intranasally and orally with the mouse *P. pneumotropica* biotype Jawetz strain NCTC 8141^T and exposed to pen-mates infected with the rat *P. pneumotropica* biotype Heyl strain SSI P331 by co-housing.

Rats were tested by PCR using primer sets developed for the specific detection of each biotype respectively.

The co-housing and exposure of rats initially infected by the mouse *P. pneumotropica* with pen-mates harbouring the rat *P. pneumotropica* led to disappearance of the mouse bacterium from the rats.

Our results question the suitability of *Pasteurellaceae* from contemporary laboratory animals to elucidate associations between bacterial properties and host species.

Introduction

Infection by *Pasteurellaceae* frequently occurs in mice and rats and bacterial strains isolated are often designated mouse and rat strains respectively. Mouse and rat *Pasteurellaceae* have been found to differ in phenotypic characteristics such as hemagglutinating activity (Boot *et al.*, 1993) and antigenic make up (Nakagawa & Saito, 1984; Boot *et al.*, 1994/96). Mouse and rat *Pasteurellaceae* and have been reported to belong to different genetic lineages (Hayashimoto *et al.*, 2006; Sasaki *et al.*, 2006a-b, 2009). Such data support the widely accepted concept of host specificity of *Pasteurellaceae* infection (Kilian & Frederiksen, 1981; Bojesen *et al.*, 2007).

Mouse *Pasteurellaceae* have however been found capable of infecting rats and vice versa (Nakagawa *et al.*, 1981; Boot *et al.*, 1994).

To bridge these conflicting findings one might speculate that although mice and rats can get infected by each other's *Pasteurellaceae*, the non-host adapted strain may be replaced by a host-adapted and better fitting strain if exposed to one such.

To evaluate this supposition we sequentially infected rats with a mouse *P. pneumotropica* and a rat *P. pneumotropica* respectively and monitored the presence of each strain by a strain-specific polymerase chain reaction (PCR) analysis.

We found that the mouse *P. pneumotropica* colonizing the rats was almost fully replaced by the subsequently infecting rat *P. pneumotropica*.

Materials and Methods

Bacterial strains

We used the mouse *P. pneumotropica* biotype Jawetz strain NCTC 8141^T and the rat *P. pneumotropica*

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biotype Heyl strain SSI P331.

Strain NCTC 8141^T is positive by PCR using the biotype Jawetz specific primer set as described by Wang *et al.* (1996). Strain SSI P331 is PCR positive using the biotype Heyl specific primer set developed by Kodjo *et al.* (1999).

We therefore designated the bacteria Ppn-M (W+) and Ppn-R (K+) respectively.

Both strains are able to colonize the rat upper respiratory tract (Boot *et al.*, 1994).

Bacterial suspensions used for experimental infection were prepared as previously described (Boot *et al.*, 1994) and standardized at about 10⁵ – 10⁶ cfu/ml using nephelometry.

Animals

Three to 4-weeks old female random bred Rivm: CpbWU rats were used.

They were obtained from a strictly barrier-maintained SPF colony, which had no evidence of *Pasteurellaceae* infection by serology, culture and PCR, nor of other (potentially) pathogenic rodent viruses, bacteria (including *Mycoplasma*) or parasites listed

in the FELASA recommendations for the monitoring of rodent colonies.

A pelleted diet for rodents (SRM-Hope Farms BV, Woerden NL) was fed *ad libitum* and tap water was available *ad libitum*.

Rats were intranasally and orally given 0.05 and 0.1 ml respectively of a bacterial suspension under KRA anesthesia [Ketamine (Alfasan, Woerden, The Netherlands) 90 mg/kg intraperitoneally (i.p.), Rompun (Bayer AG, Leverkusen, Germany) 10 mg/kg i.p., atropine (Vetinox Animal Health, Bladel, The Netherlands) 0.05 mg/kg i.p.

Experimental design

At Day 0 9 rats (group A) were dosed with the Ppn-M strain and 6 rats (group B) with the Ppn-R strain respectively (Table 1). Rats of group A and B were separately housed 3 per type III macrolon cage.

At Day 21 post infection 6 groups were formed each comprising a Ppn-M dosed rat and a Ppn-R dosed counterpart. The remaining 3 Ppn-M dosed rats were kept as controls to confirm persistent colonization of the mouse bacterium in rats.

Table 1. PCR on samples from rats experimentally infected by [*P.*] *pneumotropica*.

infected*	PCR	mouth			mouth			trachea	all samples	
		7	10	/	21	28	35	35	7-10	21-35
Ppn-M (W+)	W#	2/4	4/4	/	4/6	4/6	1/6	0/6	6/8	9/24
	K	0/4	0/4	/	6/6	6/6	5/6	6/6	0/8	23/24
Ppn-R (K+)	W	0/4	0/4	/	0/6	0/6	0/6	0/6	0/8	0/24
	K	3/4	4/4	/	6/6	6/6	4/6	5/6	7/8	21/24

Ppn-M (W+)	W	2/2	2/2	/	3/3	3/3	3/3	3/3	4/4	12/12
	K	0/2	0/2	/	0/3	0/3	0/3	0/3	0/4	0/12

* infection at D0 with either mouse [*P.*] *pneumotropica* biotype Jawetz strain NCTC 8141^T or rat [*P.*] *pneumotropica* biotype Heyl strain SSI P331, positive by PCR using a biotype Jawetz specific primer set or a biotype Heyl specific primer set, respectively. # PCR results (pos/tested) obtained using both primer sets.

Bacteriological examination

At 7 and 10 days post inoculation 4 rats of both groups A and B were tested for colonization by the bacteria given. Individual samples were obtained by flushing the mouth with 0.1 µl PBS pH 7.2 without anaesthesia. Samples were tested by PCR using the primer sets as described by Wang *et al.* (1996) and Kodjo *et al.* (1999).

At Day 21, Day 28 and Day 35 (end of study) all rats were individually sampled using mouth flushings without anaesthesia. At Day 35 all rats were sacrificed under KRA anaesthesia, examined for gross respiratory tract lesions and examined for both *P. pneumotropica* biotypes by culture and PCR using both primer sets as described (Boot *et al.*, 2009). Serology and histology were not performed. Sensitivity of the PCR methods using both primer sets was evaluated with serially diluted DNA lysates as described (Boot *et al.*, 2009) and found to range from 1 to 10 pg.

Statistics

Differences in the number of PCR positive animals were evaluated by Fisher's exact test.

Results

At 7 and 10 days post infection, PCR only detected Ppn-M (W+) and Ppn-R (K+) in mouth flushings of rats dosed with the respective bacteria (Table 1).

In the 6 groups formed at Day 21 by housing one Ppn-M dosed rat with a Ppn-R dosed counterpart, the number of rats found infected by the mouse bacterial strain gradually diminished, whereas the number of rats found infected by the rat bacterial strain gradually increased.

The remaining 3 Ppn-M dosed rats remained PCR positive for the mouse bacterium confirming persistent colonization.

Samples from Ppn-M dosed control rats were significantly more often positive by PCR using Wang's primer set than samples from rats exposed to Ppn-R rats (Fisher's exact test $p=0.005$).

In Ppn-M dosed rats exposure to Ppn-R counterparts led to a significant reduction in the number of

samples positive by PCR using Wang's primer set (Fisher's exact test $p=0.022$).

Discussion

Pasteurellaceae frequently show host specificity (Kilian & Frederiksen, 1981; Bojesen *et al.*, 2007). *Pasteurellaceae* of different types seems to prefer mice and rats, respectively, and mouse and rat *P. pneumotropica* may therefore be designated Ppn-M and Ppn-R respectively.

In the present study we found that a Ppn-M strain was able of colonizing rats, confirming earlier observations that mice and rats can be reciprocally infected with each others *P. pneumotropica* strains (Nakagawa *et al.*, 1981; Boot *et al.*, 1994).

Colonization of SPF rodents by foreign bacterial strains may partly be due to their abnormal microbial ecology as a result of rederivation, which inevitably leads to loss of the indigenous flora (Boot, Koopman & Kunstyr, 1993).

We now show that a Ppn-M strain, which is foreign to rats is almost fully replaced by a secondary infection of a Ppn-R strain (Table 1). Various mechanisms have been speculated to play a role in the between-strain competition (Hibbing *et al.*, 2009). None of these have been the subject of study in rodent *Pasteurellaceae*.

Colonization of bacteria, including *Pasteurellaceae*, in the upper respiratory tract is often mediated by fimbriae or other adhesive mechanisms (Beachy 1981; Dagmara & Czuprynski, 2009). We detected fimbriae both on Ppn-M and Ppn-R strains but also other adhesive mechanisms may be important as Ppn-M strains were hemagglutinating but Ppn-R strains were not (Boot *et al.*, 1993). It might be speculated that adhesion factors of the Ppn-R strain fit better with rat mucosal and /or cellular receptors than the Ppn-M strain does.

The fact that a secondary infection by the Ppn-R strain was accompanied by disappearance of the primary infecting Ppn-M strain from most rats (Table 1) supports the contradictory results from mutual infection studies in mice and rats (Nakagawa *et al.*, 1981; Boot *et al.*, 1994) on the one hand and host

association differences in bacteriological characteristics (for instance hemagglutinating activity) of Ppn-M and Ppn-R strains on the other hand.

It seems unlikely that a Ppn-M strain gains Ppn-R characteristics upon colonization of rats as that would involve mutation of numerous genes. In previous studies Ppn-M strains were found to ferment trehalose, but Ppn-R were not. Reciprocal infection did not lead to changes in trehalose fermenting properties (unpublished).

We studied hemagglutination and trehalose fermentation on *Pasteurellaceae* strains, notably *P. pneumotropica* obtained from 1948 to about 1970. Most of our bacterial strains likely originate from conventional rodent colonies as the first publication on the production of Specified Pathogen Free (SPF) mice appeared in 1962 (Foster, 1962) and it took several years to replace conventional animals by SPF counterparts in biomedical research.

Contemporary mice and rats usually originate from rederived barrier-maintained breeding colonies, which are often, free from *P. pneumotropica* infection, but not necessarily free from other *Pasteurellaceae*. Their use is often under less stringent hygienic conditions and *P. pneumotropica* is commonly present in experimental colonies in academia (Pritchett-Corning et al., 2009).

Studies have sought possible associations between phenotypic properties of rodent *P. pneumotropica* and their host (Nagakawa & Saito 1984; Boot et al., 1993; 1994). Recent studies focussed on possible genetic differences between mouse and rat *Pasteurellaceae* strains. Some studies showed indeed associations between bacteriological characteristics and host species (Hayashimoto et al., 2006; Sasaki et al., 2006a & 2009). Others found no association or reported bacterial clones that were shared by mice and rats (Sasaki et al., 2006a & 2009).

Unfortunately none of the studies gave details about the animal colonies from which the bacteria were obtained. So it remains unclear whether mice and rats were conventional or of SPF quality and whether mice and rats were housed within the same microbiological unit.

We studied V-factor dependent *Pasteurellaceae* (*Haemophilus* spp) from SPF guinea pigs and rats by API NH and FAME profiling and found that 66% and 76% of the guinea pig and rat *Haemophilus* strains, respectively, belonged to shared API-FAME types. The remaining strains were of types unique to either guinea pig (12 types) or rat (9 types). The bacterial strains were from breeding and experimental colonies kept according to host species (Boot, 2008).

Pasteurellaceae, mostly classified as *P. pneumotropica* have been reported from various rodent species including gerbils, hamsters and mastomys. Mutual infections are possible and a primary infecting bacterial strain may be out competed by a subsequent better fit invader. Therefore a *Pasteurellaceae* strain isolated from a SPF rat is not necessarily a 'rat-pasteurella', etc.

Our results question the suitability of *Pasteurellaceae* from contemporary laboratory animals to elucidate associations between bacterial properties and host species. It seems more reasonable to use bacterial strains obtained from for instance wild trapped rodents.

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References

- Beachy EH. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surface. J. Infect. Dis. 1981, 143, 325-345.
- Boot R, JP Koopman & I Kunstyr. Microbiological standardization. Pp 143-165 (Ch. 8) in: *Principles of Laboratory Animal Science* (LFM van Zutphen, V Baumans & AC Beynen eds.); Elsevier, Amsterdam (1993).
- Boot R, H Thuis H & JS Teppema: Haemagglutination by *Pasteurellaceae* from rodents. Zentralbl. Bakteriologie, 1993, 279, 259-273.
- Boot R, H Thuis, JL Veenema, RHG Bakker & HC Walvoort. Colonization and antibody response in

- mice and rats experimentally infected with *Pasteurellaceae* from different rodent species. Lab. Animals 1994, 28, 130-137.
- Boot R, HThuis & JL Veenema. Comparison of mouse and rat *Pasteurella pneumotropica* by micro-agglutination assay. J. Exp. Anim. Sci. 1994-96, 37, 158-165.
- Boot R, MJ Vlemminx & FAG Reubsaet. Comparison of polymerase chain reaction primer sets for amplification of rodent *Pasteurellaceae*. Lab. Animals 2009, 43, 371-375.
- Boot R. Guinea pigs and rats as carrier of host unique and shared *Haemophilus* phenotypes. Scand. J. Lab. Anim. Sci. 2008, 35, 163-167.
- Bojesen AM, H Christensen, SS Nielsen & M Bisgaard. Host-specific bacterial lineages in the taxon 2 and 3 complex of *Pasteurellaceae*. System. Appl. Microbiol. 2007, 30, 119-127.
- Dagmara IK & CJ Czuprynski. Identification of *Mannheimia haemolytica* adhesins involved in binding to bovine bronchial epithelial cells. Infect. Immun. 2009, 77, 446-455.
- Foster HL. Establishment and operation of S.P.F. colonies. Pp. 249-259 in *The Problems of Laboratory Animal Disease* (Harris, R.J.C. ed). Academic Press, London (1962).
- Hayashimoto N, M Ueno, A Takahura & T Itoh. Phylogenetic analysis of isolates of *Pasteurella pneumotropica* from laboratory animals based on the gyrB gene sequence. Exp. Animals 2006, 55, 487-490.
- Hibbing ME, C Fuqua, MR Parsek & SB Peterson. Bacterial competition: surviving and thriving in the microbial jungle. Nature Rev. Microbiol 2009, 8, 15-25.
- Kilian M & W Frederiksen. Ecology of *Haemophilus*, *Pasteurella* and *Actinobacillus*. Pp. 11-38 in: *Haemophilus, Pasteurella and Actinobacillus* (M Kilian, W Frederiksen & EL Biberstein eds). Academic Press, London-New York (1981).
- Kodjo A, L Villard, F Veillet, F Escande, E Borges, F Maurin, J Bonnod & Y Richard Identification by 16S rDNA fragment amplification and determination of genetic diversity by random amplified polymorphic DNA analysis of *Pasteurella pneumotropica* isolated from laboratory rodents. Lab. Anim. Sci. 1998, 49, 49-53.
- Nakagawa M, M Saito & K Kohjima. Mutual transmission of *Pasteurella pneumotropica* between mice and rats. Jap. J. Vet. Sci. 1981, 43, 937-40.
- Nakagawa M & M Saito. Antigenic characterization of *Pasteurella pneumotropica* strains from mice and rats. Exp. Animals 1984, 33, 187-92.
- Pritchett-Corning KR, J Cosentina & CB Clifford. Contemporary prevalence of infectious agent in laboratory mice and rats. Lab. Animals 2009, 43, 165-173.
- Sasaki H, E Kawamoto, H Ueshiba, H Amao & T Sawada. Phylogenetic relationship of *Pasteurella pneumotropica* isolates from laboratory rodents based on 16S rDNA sequence. J. Vet. Med. Sci. 2006a, 68, 639-641.
- Sasaki H, E Kawamoto, E Okiyama, H Ueshiba, K Mikazuki, H Amao & T Sawada. Molecular typing of *Pasteurella pneumotropica* isolated from rodents by amplified 16S ribosomal DNA restriction analysis and pulsed-field gel electrophoresis. Microbiol. Immunol, 2006b, 50, 265-272.
- Sasaki H, E Kawamoto, Y Tanaka, T Sawada, S Kunita & K Yagami. Comparative analysis of *Pasteurella pneumotropica* isolates from laboratory mice and rats. Antonie van Leeuwenhoek 2009, 95, 311-317.
- Wang RF, W Campbell, WW Cao, C Summage, RS Steele & CE Cerniglia. Detection of *Pasteurella pneumotropica* in laboratory mice and rats by polymerase chain reaction. Lab. Anim. Sci. 1996, 46, 81-85.