Guinea Pig and Rat as Carriers of Host-unique and Shared Haemophilus Phenotypes

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Summary

Infections by V- factor dependent *Pasteurellaceae* (commonly called *Haemophilus* spp) frequently occur in colonies of guinea pig and rat. We evaluated possible differences between 185 *Haemophilus* strains from guinea pig (n=97) and rat (n=88) by API NH biotyping and by cell wall lipid profiling (FAME-analysis). By combining results of both methods we found 28 *Haemophilus* API-FAME types. Seven API-FAME types were shared and comprised 66% and 76% of the guinea pig and rat *Haemophilus* strains respectively. The remaining 21 *Haemophilus* phenotypes were unique to either guinea pig (12 types) or rat (9 types).

Introduction

Infections by V- factor dependent *Pasteurellaceae* frequently occur in contemporary colonies of guinea pig (*Boot et al., 1996/97, 1998*) and rat (*Boot et al., 1994/95; Nicklas et al.,1993*). Infections by *Pasteurellaceae* are often host specific (*Kilian & Frederiksen, 1981*). We evaluated possible differences between *Haemophilus* strains from guinea pig and rat by API NH biotyping and by cell wall lipid profiling. We found *Haemophilus* API-FAME types shared by both animal species as well as types unique to guinea pig or rat.

Materials and Methods

Bacterial strains

The V-factor dependent *Pasteurellaceae* (*Haemophilus*) strains were cultured between 1991 and 1996 as described (*Boot et al., 1994/95*) from the respiratory tracts of healthy rats and guinea pigs from breeding and experimental colonies. The 185 *Haemophilus* strains comprised 97 strains from guinea pigs (71 from 7 breeding and 26 from 6 experimental colonies) and 88 strains from rats (35 from 6 breeding and 53 from 15 experimental colonies). Guinea pigs and rats were in breeding and experimental colonies animals housed separately.

Only in a few instances were *Haemophilus* strains obtained from a breeding colony and one or more related experimental colonies.

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Phenotypic characterization of Haemophilus

API NH profiling

All bacteria were grown on chocolate agar incubated under 5-10% CO_2 for 18 ± 1 hrs at 37 ± 0.5 °C and tested using the API NH system (Bio Merieux SA, Marcy-lÉtoile) according to the manufacturer's instructions. The system comprises tests for the fermentation of glucose, fructose, maltose and saccharose, test for activities of the enzymes ornithinedecarboxylase, urease, lipase, alkaline phosphatase, β-galactosidase, proline arylamidase and γ-glutamyltransferase, and finally the production of indole.

As results of testing for proline arylamidase and γ-glutamyltransferase did not yield reproducible results with some strains, the API NH profiles were given as a

3-digit code instead of the usual 4-digit code.

Cellular fatty acids

Profiling was carried out as described previously (*Boot et al., 1993*). Briefly bacteria were grown on GC agar base (Difco) supplemented with vitox (Oxoid) and hemoglobin (Difco) incubated under 5-10% CO_2 for 18 ± 1 hrs at 37 ± 0.5 °C.

Whole-cell fatty acids were extracted and analyzed as fatty acid methyl esters (FAMEs) and recognition of fatty acid profiles was performed with a Microbial Identification System (MIS, Microbial ID, Newark, DE USA). The quantitative data obtained from the FAME profiles were used as the basis for numerical analysis. Peak area values for each FAME were calculated as a percentage of the total peak area to eliminate the effect of inoculum size.

Statistical analysis

Differences in the frequency of *Haemophilus* types between groups were evaluated by the chi square test. The level of significance was set at $P < 0.05$.

Results

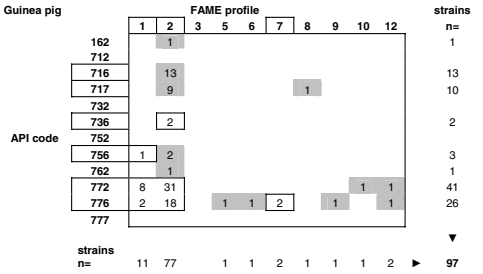
API NH profiling of the 185 *Haemophilus* strains yielded 12 API NH profiles (3 digit codes) (Table 1). Four codes were shared by *Haemophilus* strains from guinea pig and rat and comprised the majority of the *Haemophilus* strains, namely 95/97 (98%) from guinea pigs and 62/88 (70%) from rats.

Analysis of cell wall lipids as fatty acid methyl esters (FAMEs) yielded 10 FAME profiles (Table 1). Three profiles were shared by *Haemophilus* strains from both animal species and comprised the majority of the *Haemophilus* strains, namely 90/97 (93%) from guinea pigs and 87/97 (89%) from rats.

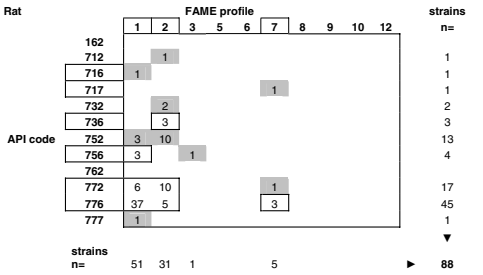
The cellular fatty acid composition of the 10 FAME profiles to which the strains could be assigned is given in Table 2. The 3 major FAMES detected in all strains were unbranched tetradecanoic acid 14:0 and the hexadecanoic acids 16:0 and 16:1 cis 9.

By combining API NH codes and FAME profiles, 28 **API-FAME** types appeared among the bacterial strains (Table 1).

Table 1. Phenotypes composed of API code and FAME profile in V-factor dependent *Pasteurellaceae* (*Haemophilus* spp) from guinea pig and rat shared types in rectangles; types unique to either guinea pig or rat shaded



13 guinea pig colonies > 19 types



16 rat colonies > 16 types

Seven API-FAME types (736-2; 756-1; 772-1&2; 776-1, 2&7) were shared by *Haemophilus* strains from both animal species. These shared types comprised the majority of the *Haemophilus* strains from guinea pig (n= 64; 66%) and rat (n= 67; 76%).

The remaining 21 types were unique to either guinea pig or rat. Guinea pig *Haemophilus* strains showed 12 unique types (of 19 detected) which comprised 33/97 (34%) of the strains from this animal species. Three unique types were represented by at least 2 strains. Rat *Haemophilus* strains showed 9 unique types (of 16 detected) which comprised 21/88 (24%) of the strains from this animal species. Three unique types were represented by at least 2 strains.

The number of host-unique *Haemophilus* strains

Table 2: Cellular fatty acids analysed as fatty acid methylesters (FAMEs) in V-factor dependent *Pasteurellaceae* (*Haemophilus* spp) from guinea pigs and rats

Fatty Acids*	FAME type # 1	2	3	5	6	7	8	9	10	12
14:0	**14.3 ± 0.9@**	**20.1 ± 1.1**	**18.5 ± 0.0**	**9.4 ± 0.0**	**23.6 ± 0.1**	**11.7 ± 0.8**	**22.1 ± 1.1**	**16.6 ± 1.3**	**21.5 ± 0.1**	**16.1 ± 0.9**
16:1 cis 9	**35.8 ± 0.7**	**32.5 ± 1.0**	**30.1 ± 0.2**	**37.4 ± 0.2**	**30.7 ± 0.1**	**36.9 ± 1.0**	**27.4 ± 0.8**	**36.8 ± 1.3**	**33.1 ± 0.2**	**37.0 ± 1.2**
16:0	**34.2 ± 1.1**	**34.9 ± 1.3**	**33.1 ± 0.3**	**23.7 ± 0.3**	**31.3 ± 0.4**	**30.6 ± 1.1**	**37.1 ± 0.7**	**32.2 ± 0.4**	**31.0 ± 0.2**	**33.0 ± 0.9**
18:0	**2.8 ± 0.7**	**1.1 ± 0.3**	**3.4 ± 0.3**	**6.1 ± 0.2**	**0.6 ± 0.0**	**4.7 ± 1.1**	**1.9 ± 0.3**	**1.7 ± 0.4**	**0.8 ± 0.1**	**3.1 ± 0.5**
sf3	**9.1 ± 0.7**	**9.2 ± 0.9**	**10.5 ± 0.0**	**9.3 ± 0.1**	**10.5 ± 0.2**	**9.5 ± 1.0**	**8.5 ± 1.0**	**8.1 ± 0.5**	**10.3 ± 0.1**	**7.7 ± 1.9**
sf7	**2.1 ± 0.3**	**0.7 ± 0.2**	**1.6 ± 0.0**	**6.8 ± 0.2**	**0.6 ± 0.0**	**3.7 ± 0.5**	**0.6 ± 0.1**	**1.7 ± 0.3**	**1.0 ± 0.0**	**2.1 ± 0.1**
strains n=	62	108	1	1	1	7	1	1	1	2
analysis n=	108	139	2	2	2	14	4	9	2	2

* summed feature (sf) 3 includes 14:0 3-OH and 16:1 iso I; sf 7 includes cis-18:1 cis 11.

@ mean ± standard deviation of mean for percentages;

profiles 4 and 11 not detected among the strains in this study

found by API NH biotyping (n = 18) and FAME analysis (n = 7) did not differ but combining the results to API-FAME types significantly increased the number of unique strains to 54 ($\chi 2 \geq 22.3$; $p < 0.001$).

The number of API-FAME types found in breeding and experimental colonies of rat and guinea pig did not differ ($\chi 2 = 6.14$; $p > 0.05$).

The frequency of occurrence of unique *Haemophilus* types and strains in guinea pig and rat did not differ ($\chi 2 = 0.002$ for types and 2.3 for strains; $p > 0.05$).

Unique types and strains were found with similar frequency in breeding and experimental colonies both in rat ($\chi 2 = 0.03$; $p > 0.05$) and guinea pig ($\chi 2 = 0.17$; $p > 0.05$).

Discussion

Genetic methodologies are at present rather popular to study the epidemiology of viral, bacterial and parasitic infections (*Wassenaar, 2003*). Phenotypic methods should however not be ignored and basically every method that yields different outcomes in a collection of bacterial strains is suitable for epidemiological studies.

To illustrate this we here summarize results of phenotypic profiling of V-factor requiring *Pasteurellaceae* (traditionally called *Haemophilus* spp) from guinea pig and rat by API NH biotyping and cell wall lipid (FAME) profiling; these data were obtained between 1991 and 1996.

API NH biotyping yielded different codes and FAME analysis yielded different profiles so both methods were basically suitable for an epidemiological evaluation of our *Haemophilus* strains. The cellular fatty acid composition of the *Haemophilus* strains (Table 2) agreed with that reported for *Pasteurellaceae* species (*Jantzen et al., 1981; Boot et al., 1993*).

As the majority of the strains from both the guinea pig and rat belonged to a small number of shared API NH codes or shared FAME profiles (Table 1), both methods seemed to be of limited value if used solely. By combining the outcomes of both methods to API-FAME types however, significantly more bacterial strains that might be unique to the guinea pig or rat were found.

The properties tested by both methods comprise biochemical processes and cell wall composition and the underlying genes will likely be dispersed over the bacterial genome. For epidemiological studies our phenotypic approach might therefore be as informative as the outcome of genetic profiling by for instance multilocus sequence typing (*Maiden, 2006*).

Our study could not indicate to which *Pasteurellaceae* species the V-factor dependent strains belong. *Brands and Mannheim (1996)* showed that various biochemical tests should be added to the API NH biotyping system for proper species classification of *Haemophilus* strains. Biochemically, V-factor-requiring *Pasteurellaceae* from rats were found

to be related to the *Haemophilus parainfluenzae*-complex (*Nicklas et al. 1993*). This is supported by 16S rDNA sequence analysis of some *Haemophilus* strains from rats (unpublished observation) but other strains from rodents have been found to belong to the Rodent cluster and Bisgaards taxon 22 (*Hayashimoto et al., 2007*).

The *H. parainfluenzae*-complex occurs in humans, the Rodent cluster in rodents and Taxon 22 is of avian origin (*Olsen, 2005*). This suggests that infection of both the guinea pig and rat originates from staff acting as a natural host or vector of the bacteria. A common source of infection is supported by finding strains with shared API-FAME types in both species, which were housed separately from each other.

The finding of host-specific API-FAME types might be considered accidental for types represented by one strain only. This seems however less likely for types that are represented by several strains. So our findings suggest that type 716-2 is better adapted to colonize the guinea pig than the rat and that the reverse applies to type 752-2 (Table 1). It could also be said that guinea pig is a better matrix / culture medium for type 716-2 than rat, and that the reverse applies to rat. Both animal species apparently form a good matrix for shared API-FAME types and will likely be mutually infective (*Boot et al., 2000*). In *Pasteurellaceae*, including *Haemophilus* species, adhesin-receptor interactions likely form the basis for differences in host tropism (*Jacques & Paradis, 1998; St Geme, 2002*).

Our data contributes to insight into the epidemiology of rodent *Haemophilus* and the frequency of occurrence of types might base the selection of strains for other studies on *Pasteurellaceae*. Bacterial typing will, irrespective of the bacterial species, be useful to trace source(s) of infection, to evaluate the effect of preventive hygienic measures and might help decide whether to keep different rodent species within the same microbiological unit (*Niklas et al., 2002*).

We conclude that most of our guinea pig and rat *Haemophilus* strains belong to a limited number of shared phenotypes identified by API NH-FAME profiling, but both animal species may harbour host-unique phenotypes as well

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