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Most European SPF 'pasteurella free' guineapig colonies are *Haemophilus* spp antibody positive

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Introduction

Based on DNA/DNA hybridization experiments the Pasteurellaceae family was established in 1981 to accomodate bacteria of the genera *Haemophilus* (then the species requiring X- (hemin or other porphyrins) and / or V- (NAD or its phosphate) factor for growth on agar), *Actinobacillus* and *Pasteurella* (*Mannheim, 1984*). As all Pasteurellaceae are obligate parasitic (*Biberstein, 1990*) and posses similar pathogenicity factors (*Nicolet, 1990*) SPF laboratory animals should be monitored and preferably found free from all members of the family. Guineapigs are mostly monitored by culture for *Pasteurella* sp., notably *Pasteurella multocida* and *Pasteurella pneumotropica*, according to the FELASA recommendations for the health monitoring of breeding colonies of rodents and rabbits (*Kraft et al., 1994*). As serology is less labour intensive than culture and can avoid the killing of animals we developed and validated ELISAs for monitoring guineapigs for antibodies to various Pasteurellaceae including V-factor dependent *Haemophilus* sp (*Boot et al., 1995a*). As we needed guineapigs without respiratory pathogenic bacteria we tested sera from various 'pasteurella free' European SPF guineapig breeding colonies for antibodies to Pasteurellaceae (and various other viral and bacterial) antigens.

Materials and Methods

In 1993 - 1998 we collected 125 sera from Dunkin Hartley guineapigs (8 - 20 weeks) present in 14 microbiological units of 8 European breeders (1 - 5 units per breeder). All units were claimed to be 'pasteurella free' (Table 1) based on cultural examination. Sample size in our study was 5 to 15. Some units were tested twice or more, but only the data obtained with the 1st sample were included in this paper.

Sera were tested by ELISA using antigens of 4 growth factor independent Pasteurellaceae (Table 1): *Actinobacillus*-like taxon 5 (strain Tax 5, guineapig origin), *P. multocida* capsule type A (strain Pm A, rabbit origin), *P. pneumotropica* (strain NCTC 8284, mouse origin) and SP group pasteurella (strain Pg 28, guineapig origin) (*Boot et al., 1995a*) and 2 V-factor requiring *Haemophilus* strains: H21 (rat origin; *Boot et al., 1994/5*) and H35 from a guineapig (*Boot et al., 1999*).

Negative and positive control sera were run in each test. Negative control sera were from hysterectomy-derived Pasteurellaceae free 2/N guineapigs maintained under gnotobiotic conditions in Gustaffson type isolators. Positive sera were raised by immunization of isolator kept counterparts as described (*Boot et al., 1995a*). All sera were tested in duplicate in the 1 : 50 dilution. Sera were considered as positive if the optical density (OD) value of a serum dilution exceeded the mean + 3 SD of the mean of the ODs in the negative control sera and 30 % of the OD of the positive control serum.

Samples from the respiratory tract of guineapigs from microbiological units 1, 7, 13 and 14 were incubated on (horse blood based) chocolate agar (CA) and on CA containing 2 µg / ml of clindamycin HCl to detect growth factor requiring bacteria (*Haemophilus* sp) and on plain sheep blood agar (SBA) and SBA containing 2 µg / ml of clindamycin HCl (SBA-cl) to detect growth factor

Table 1. Antibody activity against 6 Pasteurellaceae antigens in guineapigs from 14 SPF 'pasteurella free' microbiological units of 8 European breeders (1993 - 1998).

Microb. unit	Breeder	Unit	Tested	Tax 5 *	H21	H35	PmA	Ppn	SP	pos tests (%)
1	A		5	1**	1	4	0	1	0	7 (23)
2	B	1	10	2	3	0	0	2	0	7 (12)
3		2	10	2	0	0	0	0	0	2 (3)
4	C		10	10	10	10	8	10	10	58 (97)
5	D	1	10	9	8	9	2	7	7	42 (70)
6		2	10	9	4	7	0	1	2	23 (38)
7	E		10	0	2	0	0	0	0	2 (3)
8	F	1	5	0	0	1	0	0	0	1 (3)
9		2	10	4	9	6	1	5	3	28 (47)
10		3	5	1	2	2	0	2	1	8 (27)
11		4	5	0	0	0	0	1	0	1 (3)
12		5	15	13	15	15	3	14	13	73 (81)
13	G		10	0	0	2	0	0	0	2 (3)
14	H		10	6	9	6	1	4	1	27 (45)
units	pos	n		10	10	10	5	10	7	
		=								
		%		71	71	71	36	71	50	
sera	pos	n		57	63	62	15	47	37	281
		=								(37)
		%		46	50	50	12	38	30	

* Tax 5: *Actinobacillus*-like taxon 5; H21; *Haemophilus* sp strain H21 (rat); H35: *Haemophilus* sp strain H35 (guineapig); PmA: *Pasteurella multocida* capsule type A (rabbit); Ppn: *Pasteurella pneumotropica* (mouse); SP: SP group pasteurella sp (guineapig)
** number of positive sera.

independent strains as described previously (*Boot et al., 1994/5*). Guineapigs from other colonies were not cultured.

Data analysis

The χ^2 test was used to evaluate possible differences in the number of seropositive units found by the different ELISAs and in the total number of guineapigs found seropositive by ELISAs using *Haemophilus* and non-*Haemophilus* antigens respectively. When sera from a unit showed antibody activity against more than one Pasteurellaceae antigen, sample ODs obtained in the ELISAs were expressed as % of the OD of the corresponding positive control serum. Then we tested possible differences in median OD values calculated for the ELISAs per unit using the Wilcoxon test. Correlation of antibody activity (sample OD as % of positive control) measured by the various ELISAs was further evaluated by calculating Kendalls rank correlation coefficient and associated Z values. P values < 0.05 were considered to indicate significant differences and correlations respectively. Test were done using the

SYSTAT statistical package run on a personal computer.

Results

Seropositive guineapigs were detected in samples from all 14 breeding units examined (Table 1). Five colonies tested positive in a single ELISA: unit 3 by *Actinobacillus*- like taxon 5, unit 7 by *Haemophilus* H21, units 8 and 13 by *Haemophilus* H35 and unit 11 by *P. pneumotropica* antigen. Four units (4, 5, 9 and 14) tested positive by all 6 antigens. Different units of the same breeder contained guineapigs that tested similarly (breeder 4) or dissimilarly (breeders 2 and 6). Breeder 6 appeared to have 2 units that tested positive with a single antigen and 2 units that tested positive with all 6 antigens. The percentage of positive tests ranged between 3 and 97 (Table 1).

The number of units tested positive by the various ELISAs did not differ significantly (χ^2= 7.1, p = 0.22, df 5). ELISAs performed with *Haemophilus* H21 and H35 antigens were significantly more often positive than ELISAs performed with non-*Haemophilus* antigens: 125 of 250 tests versus 156 of 500 tests (χ^2 = 24.3, p < 0.001, df = 1). The P. *multocida* ELISA detected significantly fewer positive sera than the other assays done with non-*Haemophilus* antigens (χ^2 = 58.2, p < 0.001, df = 5). In some units in which guineapigs showed antibody activity against more than one antigen, antibody activities (sample OD expressed as % of positive control) measured by both *Haemophilus* ELISAs were correlated and occasionally correlations between activity against *Haemophilus* antigens and non-*Haemophilus* antigens were found (data not shown). Median antibody levels detected by *Haemophilus* ELISAs were mostly significantly higher than median antibody levels measured by the other ELISAs (Table 2; Wilcoxon test, p < 0.05), but differences did not reach significancy when only 5 sera were tested.

Culture of the respiratory tracts of guineapigs from units 1, 7, 13, 14 (animals from other units not cultured) detected V factor requiring Pasteurellaceae (*Haemophilus* sp) only (data not shown). The bacteriological and serological properties of some isolates have been described (*Boot et al., 1999*).

Table 2. Median antibody activity to Pasteurellaceae antigens in ELISA positive guineapigs

Microb. unit	Breeder	Unit	Tested	Tax 5*	H21	H35	PmA	Ppn	SP
1	A		5	5	9.5	**20****	7.5	4	8
2	B	1	10	7	**34**	6	6	10	7
4	C		10	71	52.5	**83**	34.5	63	63.5
5	D	1	10	45.5	45.5	**56.5**	12	35.5	44.5
6		2	10	42	30	**64**	9.5	25.5	27.5
9	F	2	10	26.5	**44.5**	37	13.5	29	17
10		3	5	16	26	24	19	26	25
12		5	15	49	**98**	73	28	63	47
14	H		10	36.5	**59.5**	32.5	12	29.5	19.5

* see Table 1

** sample OD as % of positive control. In bold: major antibody respons to *Haemophilus* sp.

Discussion

Several Pasteurellaceae have been reported from pneumonic guineapigs (references in *Boot et al., 1995a*) and in our laboratory ELISAs have been set up for the detection of antibodies to non V- or X- factor requiring species (*P. multocida*, *P. pneumotropica*, *Actinobacillus* - like taxon 5 and the SP group pasteurella) and 2 V- factor dependent bacteria, traditionally called *Haemophilus* sp. Our serological findings using ELISA antigens of these bacteria (Table 1) suggest that Pasteurellaceae infection was present in all 'pasteurella free' guineapig breeding units tested.

As both *Haemophilus* sp antigens detected significantly more positive samples than each of the other antigens (Table 1), and in most units the median antibody activity was highest with an *Haemophilus* antigen (Table 2), *Haemophilus* sp infection seems to be most likely. *Haemophilus* infection was confirmed in guineapigs from all 4 units examined by culture (data not shown). In one of these (unit 3) antibodies were detected by the Actinobacillus-like taxon 5 ELISA only, stressing the existence of complex serological relationships among Pasteurellaceae. Although infection by growth factor independent Pasteurellaceae can not be fully excluded, *P. multocida* and *P. pneumotropica*, if present, would likely have been detected by the breeder. Colony morphology and biochemical characteristics however of *Actinobacillus*-like taxon 5, the SP group pasteurella and *Pasteurella*-like taxon 6 bacteria, differ from both well known *Pasteurella* species (*Boot and Bisgaard, 1995b*) and hence isolates might have been overlooked or discarded.

Breeding colonies of guineapigs do not seem to be monitored for *Haemophilus* infection or, if monitored, results may not be reported, as FELASA recommends to monitor breeding colonies for *Pasteurella* species infection only (*Kraft et al., 1994*). As all Pasteurellaceae are parasitic and must be considered of similar importance to laboratory animals (*Biberstein, 1990*) FELASA's recommendation to monitor experimental colonies for all Pasteurellaceae (*Rehbinder et al., 1996*) is justified. We conclude that SPF guineapigs claimed to be 'pasteurella free' are likely infected by V - factor requiring Pasteurellaceae commonly called *Haemophilus* sp.

Summary

During 1993 - 1998 we tested sera of 'pasteurella free' guineapigs from 14 SPF breeding units of 8 European breeders by ELISA using whole cell antigens of 4 growth factor independent Pasteurellaceae (*Actinobacillus*-like taxon 5, *P. multocida*, *P. pneumotropica* and SP group pasteurella) and 2 V - factor requiring Pasteurellaceae (*Haemophilus* sp). Seropositive guineapigs were detected in all 14 breeding units. The ELISAs performed with *Haemophilus* antigens detected significantly more positive samples than ELISAs done with non-*Haemophilus* antigens. In most units showing antibody activity against more than one Pasteurellaceae antigen, median antibody levels detected by *Haemophilus* ELISAs were significantly higher than levels measured by the other assays. In 4 colonies also examined by culture the serological findings were confirmed by growth of *Haemophilus* sp, but growth factor independent Pasteurellaceae were not detected. Our findings indicate that European 'pasteurella free' guineapig breeding colonies are very likely infected by V-factor dependent Pasteurellaceae (*Haemophilus* sp).

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