Rat Strains Differ in Antibody Response to *Streptobacillus moniliformis*

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Summary

Rats are the natural host of *Streptobacillus moniliformis*. The bacterium is zoonotic which is an obvious reason for periodic monitoring of rat colonies for *S. moniliformis* infection. This may be done by measuring antibodies to the bacterium by for instance the enzyme-linked immunosorbent assay (ELISA). Inbred rat strains can significantly differ in antibody response to viral, bacterial and parasitic infections. We tested the hypothesis that in rats the antibody response to *S. moniliformis* is under genetic control. Rats of 6 inbred strains were intranasally and orally dosed with the bacterium and monitored for the development of antibodies by ELISA.

Rats of all strains rapidly seroconverted to the bacterium. The rat strains differed significantly in final antibody activities and seroconversion rates. WKY and SHR rats were high responders, BN and F344 rats were low responders and LEW and BD rats were intermediate both in antibody development and seroconversion.

Introduction

Streptobacillus moniliformis is a Gram negative bacterium found in various laboratory animal species and is the causative agent of rat bite fever and Haverhill fever in man (*Elliott 2007; Gaastra et al., 2009*). Since 1985 *S. moniliformis* infection has been occasionally detected in SPF laboratory rodent colonies (*Boot et al., 2002*) and although the infection seems to be rare, the detection of this zoonotic infection inevitably leads to eradication measures being required. Periodic monitoring of rodent colonies for *S. moniliformis* is recommended by FELASA (*Nicklas et al., 2002*). This is traditionally carried out by culture but culture of *S. moniliformis* from asymptomatic animals is notoriously difficult. The bacteria may also be detected by PCR (*Boot et al., 2002*). For large-scale screening purposes it is convenient to measure antibodies to the bacterium by for instance an enzyme-linked immunosorbent assay (ELISA) (*Boot et al., 1993*) and then use PCR for confirmation.

Recently we described that rat strains differ in antibody response to *Haemophilus* species infection (*Boot et al., 2005*). To test the hypothesis that in rats also the antibody response to *S. moniliformis* is under genetic control we monitored the development of antibodies in 6 inbred strains of rats after experimental infection.

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Materials & Methods

Ethical note

The protocol of the experiments was peer-reviewed by the Scientific Committee and approved by the Animal Experiments Committee of the National Institute of Public Health. Animal experiments were

conducted in compliance with national legislation which is based on European Community directive 86/609/EEC.

Animals

Six female rats aged 4 to 5 weeks of each of the following 6 strains were used: BDIX/OrlIco (BDIX), BN/OrlIco (BN), F344/Ico (F344), LEW/NIco (LEW), WKY/NIco (WKY), and SHR/NCrl (SHR). The rats were from 3 different barrier-maintained SPF breeding units of the same supplier and were reported to be free from inadvertent rodent infections listed in the FELASA recommendations for the monitoring of colonies of rodents and rabbits (*Nicklas et al., 2002*).

Housing conditions

The experiment was carried out under strict containment conditions in a Gustafsson type stainless steel isolater. Per strain the rats were housed 3 per type III Macrolon® cage. From 3 weeks prior to the *S. moniliformis* infection the rats were twice a week exposed to bedding from other cages in order to obtain a similar background flora in all rat strains at the start of the experiment.

Feeding and watering

The animals were fed 2.5 Mrad gamma-irradiated pelleted diet *ad libitum* (SMR, Hope Farms, Woerden NL). Tap water was available *ad libitum*.

Bacteria

S. moniliformis strain KUN-3 was grown on 5% sheep blood agar (SBA) (Oxoid) for 48 h at 37 °C under 7.5% CO_2.

Experimental infection

All rats were deliberately dosed nasally (0.1 ml) and

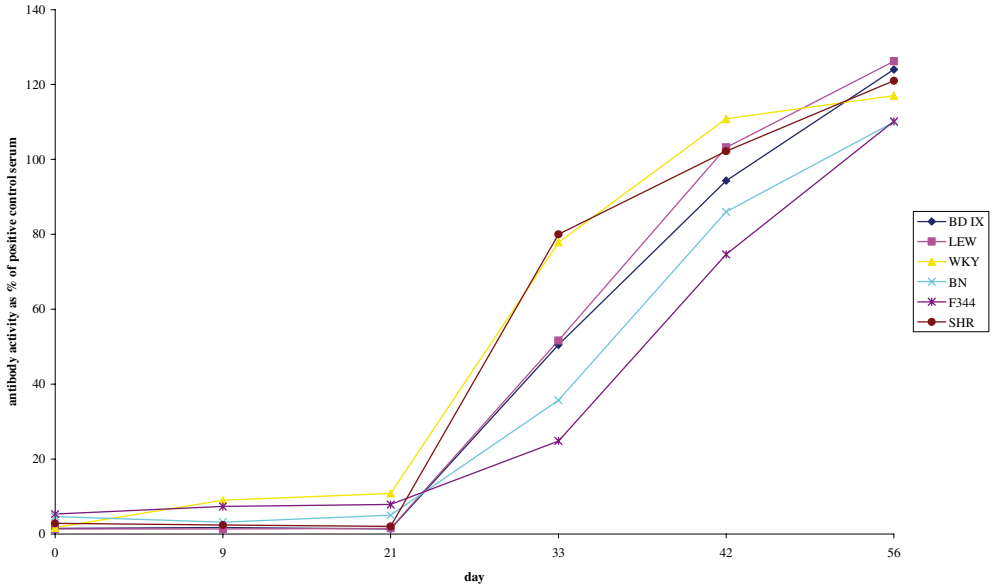

Figure 1. Antibody development to *S. moniliformis* in 6 inbred rat strains after oral and intranasal infection on day 21.
Differences in response among the strains measured at day 33 in 1:50 diluted sera diminish after day 42 suggesting test limitations.

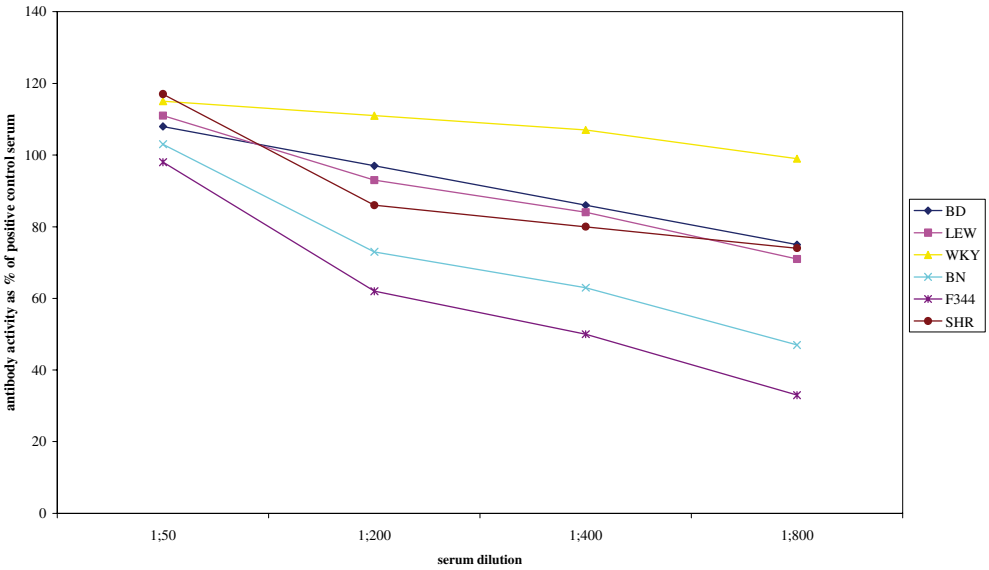

Figure 2. Differences in antibody activity to *S. moniliformis* in 6 inbred rat strains measured on day 33 are masked on day 42 in 1:50 diluted sera and reappear at higher serum dilutions.

orally (0.1 ml) with a suspension containing 10^4 to 10^5 cfu *S. moniliformis* strain KUN-3.

Detection of S. moniliformis infection

Blood samples were obtained by orbital puncture under KRA [Ketamine (Alfasan, Woerden NL) 90 mg/kg i.p., Rompun (Bayer AG, Leverkusen Germany) 10 mg/kg i.p., atropine (Vetinex Animal Health, Bladel NL) 0.05 mg / kg i.p.) anesthesia from all rats 3 weeks prior to infection (day 0), at day 9, just before *S. moniliformis* was dosed (day 21), at 12 (day 33), 21 (day 42) and 35 (day 56) days *post infectionem* (p.i.) when they were sacrificed. Sera were prepared and kept at -20 °C until testing.

Antibodies to *S. moniliformis* strain KUN-3 were determined by ELISA using sheep anti-rat IgG (Cappel, Malvern USA) as the secondary antibody as described (*Boot et al., 1993*).

Sera obtained at days 0, 9, 21, and 33 (12 days p.i.) were tested at a dilution 1: 50.

At this dilution obvious differences in antibody response among the strains were found at day 33, but less in sera taken later (Fig. 1), suggesting test limitations due to shortage of secondary antibodies (*Butler 2000*). Therefore sera obtained at day 42 (21 days p.i.) and day 56 (35 days p.i.) were also tested at higher dilutions (Table 2).

To confirm the presence of *S. moniliformis* 3 rats per strain were examined. Moistened pharyngeal swabs were inoculated onto 20% horse serum agar made selective by the addition of 16 g/l trimetroprim (ICN Biochemicals, Zoetermeer NL) and 304 g/l sulfamethoxazole (Sigma Aldridge, Zwijndrecht NL). Agar plates were incubated 48 h at 37 °C under 7.5% CO_2 and read. *S. moniliformis* suspected growth was pure cultured and identified by PCR (*Boot et al., 2002*). The remaining material on the swab was suspended in PBS pH 7.2 and tested by PCR (*Boot et al., 2002*).

All rats were examined for gross lesions but histopathologic studies were not carried out.

Statistical analysis

ELISA activity (OD) was expressed as a percent-

age of the positive control serum (OD sample/OD C+)*100 % (in short S/C+ %) and ≥ 30% was considered a positive result.

As the uninfected (and seronegative) rat strains slightly differed in background (day 0) ELISA activity, absolute and relative change in antibody activity (seroconversion) was calculated from S/C+ % data measured in sera taken at Days 0 and 33.

All statistical analyses were carried out according to recognised protocols (Petri & Watson, 1999; Quinn

Table 1. ELISA antibody activity (on days 0, 9, 21, and 33) to *S. moniliformis* antigen in rats of six inbred strains after oral and intranasal inoculation on day 21.

(a)

Parameter / Time point	**Contrast significance**[3]	**Inbred strain**						**ANOVA/Fisher's exact test significance**[4]
		BDIX	**LEW**	**WKY**	**BN**	**F344**	**SHR**	
Antibody activity (mean ± SD)[2]	**Strain→ Day↓**	αβγδ	εζηθ	αε	βζ	γη	δθ	
Day 0	A	1.5 ± 0.8[a5]	1.3 ± 0.5[b]	1.7 ± 0.5[c]	4.7 ± 5.1	5.3 ± 2.7	2.8 ± 0.4[abc]	**0.000**[6]
Day 9	B	1.7 ± 0.5	1.3 ± 0.5[a]	9.0 ± 17.2	3.2 ± 2.9	7.3 ± 9.2	2.3 ± 0.5[a]	**0.013**[6]
Day 21	C	1.3 ± 0.5	1.5 ± 0.8	10.8 ± 16.3	5.0 ± 5.7	7.8 ± 8.9	2.0 ± 0.6	**0.001**[6]
Day 33	ABC	50.5 ± 8.0[ab]	51.7 ± 6.3[cde]	77.8 ± 6.3[acf]	35.7 ± 15.1[f]	24.8 ± 11.9[adg]	80.0 ± 10.9[befg]	**0.000**
Seroconversion (change, mean ± SD)								
Absolute, day 33 - day 0		49.0 ± 7.8*[ab]	50.3 ± 6.1*[cd]	76.2 ± 6.1*[ace]	31.0 ± 14.2*[ef]	19.5 ± 10.3*[ad]	77.2 ± 10.8*[bdf]	**0.000**
Relative, ([day 33 - day 0]/day 0)		39.1 ± 16.2*[ab]	41.6 ± 13.2*[cd]	50.3 ± 18.6*[ef]	10.1 ± 6.6*[aceg]	4.2 ± 2.2*[bdfh]	27.7 ± 5.4*[gh]	**0.000**[6]
Number of positive animals								
Day 0		0	0	0	0	0	0	1.000
Day 9		0	0	1	0	0	0	1.000
Day 21		0	0	1	0	0	0	1.000
Day 33		6	6	6	5	2	6	**0.007**

ELISA = enzyme-linked immunosorbent assay.

1 Values on days 0, 9, 21, and 33 are for six animals per strain. Sera were diluted 1:50.

2 (Ranked-transformed) *antibody activity* was analyzed with multivariate repeated measures ANOVA with between-subject factor *strain* and within-subject factor *day*. There were significant *strain* (P = 0.000), *day* (P = 0.000), and *interaction* (P = 0.000) effects.

3 The multivariate repeated measures ANOVA was performed utilizing simple contrasts to compare either the six strains or the four days. Within a row (*strain*) or a column (*day*) values bearing the same greek (*strain*) or capital (*day*) letter are significantly (*strain* contrasts: $P < 0.01$; *day* contrasts: $P < 0.017$) different.

4 P value in one-way ANOVA (mean ± SD) or Fisher's exact test (number of positive animals). Significant *strain* effects ($P < 0.05$) are indicated in bold characters.

5 Post hoc testing was done by unpaired Student's t test (mean ± SD, homoscedasticity), unpaired Student's t test with Welch-Satterthwaite correction (mean ± SD, heteroscedasticity), or Fisher's exact test (number of positive animals). Within a row values bearing the same superscript letter are significantly different ($P < 0.01$).

6 P value after logarithmic transformation of the data.

7 P values; significant differences are indicated in bold characters.

* Change significantly different from zero ($P < 0.05$; Student's one-sample t test for paired data).

(b)

	Comparisons														
Parameter / Time point	**BDIX *versus* LEW**	**BDIX *versus* WKY**	**BDIX *versus* BN**	**BDIX *versus* F344**	**BDIX *versus* SHR**	**LEW *versus* WKY**	**LEW *versus* BN**	**LEW *versus* F344**	**LEW *versus* SHR**	**WKY *versus* BN**	**WKY *versus* F344**	**WKY *versus* SHR**	**BN *versus* F344**	**BN *versus* SHR**	**F344 *versus* SHR**
Antibody activity (mean ± SD)															
Contrasts (strain)	0.651[7]	**0.000**	0.344	0.059	**0.000**	**0.000**	0.180	0.027	**0.000**	**0.000**	**0.000**	0.102	0.321	**0.003**	0.034
Post hoc comparisons (strain)															
Day 0	0.687	0.687	0.163	0.015	**0.006**	0.290	0.141	0.013	**0.000**	0.181	0.019	**0.001**	0.782	0.419	0.069
Day 9	0.290	0.343	0.245	0.190	0.049	0.324	0.162	0.170	**0.007**	0.431	0.838	0.385	0.313	0.508	0.239
Day 21	0.687	0.213	0.147	0.134	0.075	0.221	0.167	0.114	0.270	0.428	0.701	0.243	0.526	0.228	0.170
Day 33	0.785	**0.000**	0.059	**0.001**	**0.000**	**0.000**	0.037	**0.001**	**0.000**	**0.000**	**0.000**	0.682	0.197	**0.000**	**0.000**
Seroconversion (change, mean ± SD)															
Absolute, day 33 - day 0															
Post hoc comparisons (strain)	0.748	**0.000**	0.021	**0.000**	**0.000**	**0.000**	0.012	**0.000**	**0.000**	**0.000**	**0.000**	0.847	0.139	**0.000**	**0.000**
Relative, ([day 33 - day 0]/day 0)															
Post hoc comparisons (strain)	0.780	0.293	**0.002**	**0.003**	0.151	0.370	**0.001**	**0.001**	0.051	**0.002**	**0.002**	0.030	0.084	0.000	**0.000**
Number of positive animals															
Post hoc comparisons (strain)															
Day 33	1.000	1.000	1.000	0.061	1.000	1.000	1.000	0.061	1.000	1.000	0.061	1.000	0.242	1.000	0.061

(c)

	Comparisons					
Parameter	**Day 0 *versus* Day 9**	**Day 0 *versus* Day 21**	**Day 0 *versus* Day 33**	**Day 9 *versus* Day 21**	**Day 9 *versus* Day 33**	**Day 21 *versus* Day 33**
Antibody activity (mean ± SD)						
Contrasts (day)	0.857	0.230	**0.000**	0.413	**0.000**	**0.000**

& Keough, 2002; Field 2009) using a SPSS® for Windows (version 16.0) computer program (SPSS Inc., IL, USA). Two-sided, exact (i.e. for the non-parametric tests; Munday & Fischer 1998) probabilities were estimated throughout. The antibody activity and seroconversion data are continuous data and were described by means and standard deviations (SD). The Kolmogorov-Smirnov one-sample test was used to check Gaussianity of the continuous data. This was done per group (i.e. per strain, day and dilution-combination) and led to the conclusion that the continuous results were normally distributed.

The significance of differences between strains (Tables 1 and 2), days (Tables 1 and 2) and dilutions (Table 2) was calculated with analyses of variance (ANOVA's). For all ANOVA's, homoscedasticity was tested by the Levene's test, which is a powerful and robust test based on the F statistic (*Lim & Loh, 1996*). When necessary, the variances were equalized by logarithmic transformation of the continuous data. After logarithmic transformation, the variances were similar and the transformed within-group data were still normally distributed. If it was not possible to fulfill this criterion via logarithmic transformation, the continuous parameter in question was rank-transformed (*Conover & Iman, 1981*).

The antibody activity data were subjected to multivariate repeated measures ANOVA with strain as main (between-subject) factor and time (day) and/or - in case of Table 2 - dilution as within-subject factors. Tests of significance are derived using the Wilk's lambda criterion. The choice of a multivari-

ate instead of a univariate statistic in the repeated measures ANOVA is based on the criteria given by Algina & Keselman (*1997*). The multivariate repeated measures ANOVA were performed utilizing

Table 2. ELISA antibody activity (on days 42 and 56) to *S. moniliformis* antigen in rats of six inbred strains after intranasal inoculation on day 21[1]

(a)

Parameter / Time point	Dilution	Contrast significance[5]	Inbred strain BDIX	LEW	WKY	BN	F344	SHR	ANOVA significance[6]
Antibody activity (mean ± SD)[2]									
		Strain→ Dilution↓	αβ	γδ	αγε	εζ	αδη	βζη	
Day 42[3]	*1:50*	A	107.7 ± 5.2	111.0 ± 5.6	114.5 ± 8.1	103.2 ± 16.4	97.5 ± 14.4	116.7 ± 8.9	**0.043**
	1:200	A	97.3 ± 7.4^{ab7}	92.6 ± 6.8^{c}	110.8 ± 6.1^{abcd}	72.8 ± 13.3^{a}	62.0 ± 10.5^{bce}	86.3 ± 9.6^{de}	**0.000**
	1:400	A	86.0 ± 9.7^{ab}	84.2 ± 9.1^{c}	107.0 ± 9.4^{abcd}	63.0 ± 12.9^{a}	49.7 ± 6.1^{bce}	79.5 ± 7.7^{de}	**0.000**
	1:800	A	75.2 ± 12.4^{a}	70.6 ± 13.6^{b}	99.3 ± 10.6^{abc}	46.7 ± 9.5^{ab}	33.2 ± 4.5^{abc}	73.5 ± 7.2^{c}	**0.000**
		Strain→ Dilution↓	αβ	γδ	αγε	εζ	αδη	βζη	
Day 56[4]	*1:50*	A	124.0 ± 5.6^{ab}	126.2 ± 10.9^{c}	117.0 ± 6.1	110.0 ± 6.1^{a}	110.2 ± 4.9^{bce}	121.0 ± 5.2^{e}	**0.001**
	1:400	A	80.5 ± 9.1^{ab}	87.0 ± 5.2^{c}	100.0 ± 3.2^{acd}	80.7 ± 11.3^{d}	73.5 ± 6.6^{ce}	98.2 ± 5.5^{be}	**0.000**
	1:800	A	68.0 ± 5.4^{ab}	73.8 ± 8.6^{c}	94.2 ± 4.6^{acd}	66.5 ± 15.9^{d}	57.7 ± 6.8^{ce}	91.4 ± 8.2^{be}	**0.000**
	1:1600	A	54.7 ± 11.0^{ab}	58.0 ± 4.5^{cd}	81.8 ± 6.7^{ace}	48.3 ± 14.1^{ef}	39.5 ± 7.7^{cd}	74.0 ± 7.2^{bdf}	**0.000**
	1:3200	A	38.3 ± 11.3^{a}	35.8 ± 4.8^{bc}	62.8 ± 9.5^{abd}	30.0 ± 11.3^{de}	22.7 ± 7.4^{bf}	53.8 ± 8.6^{cef}	**0.000**

ELISA = enzyme-linked immunosorbent assay.

1 Values on day 42 are for five (LEW, one animal died) or six animals per strain (BDIX, WKY, BN, F344, SHR). Values on day 56 are for five (LEW, one animal died; SHR, for one animal there was no day 56 serum available) or six animals per strain (BDIX, WKY, BN, F344).

2 A*ntibody activity* was analyzed with multivariate repeated measures ANOVA with between-subject factor *strain* and within-subject factors *day* and *dilution* (1:50, 1:400, and 1:800). There were significant *strain* ($P = 0.000$), *day* ($P = 0.000$), *dilution* ($P = 0.000$), and *interaction* (*strain* x *day*: $P = 0.006$, *strain* x *dilution*: $P = 0.000$, *strain* x *day* x *dilution*: $P = 0.012$) effects. The *day* x *dilution* – *interaction* effect was non-significant ($P = 0.857$).

3 For day 42 *antibody activity* was analyzed with multivariate repeated measures ANOVA with between-subject factor *strain* and within-subject factor *dilution* (1:50, 1:200, 1:400, and 1:800) There were significant *strain* ($P = 0.000$), *dilution* ($P = 0.000$), and *interaction* (*strain* x *dilution*: $P = 0.000$) effects.

4 For day 56 *antibody activity* was analyzed with multivariate repeated measures ANOVA with between-subject factor *strain* and within-subject factor *dilution* (1:50, 1:400, 1:800, 1:1600, and 1:3200) There were significant *strain* ($P = 0.000$), *dilution* ($P = 0.000$), and *interaction* (*strain* x *dilution*: $P = 0.000$) effects.

5 The multivariate repeated measures ANOVA was performed utilizing simple contrasts to compare either the six strains or the four (day 42) or five (day 56) dilutions. Within a row (*strain*) or a column (*dilution*) values bearing the same greek (*strain*) or capital (*dilution*) letter are significantly (*strain* contrasts: $P < 0.01$; *dilution* contrasts: day 42, $P < 0.017$; day 56, $P < 0.013$) different.

6 *P* value in one-way ANOVA. Significant *strain* effects ($P < 0.05$) are indicated in bold characters.

7 Post hoc testing was done by unpaired Student's *t* test (mean ± SD, homoscedasticity) or unpaired Student's *t* test with Welch-Satterthwaite correction (mean ± SD, heteroscedasticity. Within a row values bearing the same superscript letter are significantly different ($P < 0.01$).

(b)

		Contrasts														
		BDIX versus	***BDIX versus***	***BDIX versus***	***BDIX versus***	***BDIX versus***	***LEW versus***	***LEW versus***	***LEW versus***	***LEW versus***	***WKY versus***	***WKY versus***	***WKY versus***	***BN versus***	***BN versus***	***F344 versus***
Parameter	***Dilution***	***LEW***	***WKY***	***BN***	***F344***	***SHR***	***WKY***	***BN***	***F344***	***SHR***	***BN***	***F344***	***SHR***	***F344***	***SHR***	***SHR***
Antibody activity (mean ± SD) – Day 42																
Contrasts (strain)		0.688	**0.001**	**0.000**	**0.000**	0.581	**0.001**	**0.001**	**0.000**	0.901	**0.000**	**0.000**	**0.000**	0.024	**0.001**	**0.000**
Post hoc comparisons (strain)																
	1:50	0.335	0.113	0.538	0.153	0.058	0.436	0.339	0.075	0.250	0.161	0.030	0.668	0.540	0.107	0.020
	1:200	0.301	**0.006**	**0.003**	**0.000**	0.050	**0.001**	0.015	**0.000**	0.253	**0.000**	**0.000**	**0.000**	0.149	0.072	**0.002**
	1:400	0.760	**0.003**	**0.006**	**0.000**	0.228	**0.003**	0.013	**0.000**	0.379	**0.000**	**0.000**	**0.000**	0.055	0.027	**0.000**
	1:800	0.574	**0.005**	**0.001**	**0.000**	0.782	**0.003**	**0.007**	**0.002**	0.661	**0.000**	**0.000**	**0.001**	**0.010**	**0.000**	**0.000**
Antibody activity (mean ± SD) – Day 56																
Contrasts (strain)		0.488	**0.000**	0.160	**0.006**	**0.002**	**0.002**	0.047	**0.001**	0.017	**0.000**	**0.000**	0.430	0.135	**0.003**	**0.000**
Post hoc comparisons (strain)																
	1:50	0.675	0.066	**0.002**	**0.001**	0.388	0.110	0.012	**0.010**	0.365	0.076	0.058	0.279	0.960	0.012	**0.006**
	1:400	0.194	**0.001**	0.978	0.159	**0.004**	**0.001**	0.280	**0.005**	0.011	**0.007**	**0.000**	0.515	0.209	0.012	**0.000**
	1:800	0.205	**0.000**	0.832	0.016	**0.000**	**0.001**	0.384	**0.007**	0.011	**0.002**	**0.000**	0.498	0.240	0.012	**0.000**
	1:1600	0.519	**0.000**	0.405	0.020	**0.008**	**0.000**	0.162	**0.001**	**0.003**	**0.000**	**0.000**	0.095	0.207	**0.005**	**0.000**
	1:3200	0.635	**0.002**	0.231	0.018	0.034	**0.000**	0.315	**0.008**	**0.003**	**0.000**	**0.000**	0.135	0.213	**0.004**	**0.000**

(c)

	Contrasts												
	1:50 versus	***1:50 versus***	***1:50 versus***	***1:50 versus***	***1:50 versus***	***1 :200 versus***	***1:200 versus***	***1:400 versus***	***1:400 versus***	***1:400 versus***	***1:800 versus***	***1:800 versus***	***1:1600 versus***
Parameter	***1:200***	***1:400***	***1:800***	***1:1600***	***1:3200***	***1:400***	***1:800***	***1:800***	***1:1600***	***1:3200***	***1:1600***	***1:3200***	***1:3200***
Antibody activity (mean ± SD) – Day 42													
Contrasts (dilution)	**0.000**	**0.000**	**0.000**	-	-	**0.000**	**0.000**	**0.000**	-	-	-	-	-
Antibody activity (mean ± SD) – Day 56													
Contrasts (dilution)	-	**0.000**	**0.000**	**0.000**	**0.000**	-	-	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

simple contrasts to compare the six strains (Tables 1 and 2), the four days (Table 1), or the four/five dilutions (Table 2). Seroconversion, as well as antibody activity per day (Table 1) and dilution (Table 2), was subjected to a one-way ANOVA with strain as between-subject factor.

Post hoc strain comparisons were done with unpaired Student's *t* tests. The unpaired Student's *t* tests were performed using pooled (for equal variances) or separate (for unequal variances) variance estimates. The equality of variances was tested with the Levene's test. For the unpaired Student's *t* test with separate variance estimates, SPSS® uses the Welch-Satterthwaite correction (*Ruxton 2006*). Student's one-sample *t* test for paired data was used to evaluate if the seroconversion within a strain was significantly different form zero.

The statistical significance of differences between strains (per day) for the discrete data (numbers of positive animals) was calculated using the Fisher's exact test. For these data, *post hoc* strain comparisons were also done with the Fisher's exact test.

To take the greater probability of a Type I error due to multiple hypotheses (i.e. the simple contrasts and the *post hoc* strain comparisons) into account, a more stringent criterion should be used for statistical significance for the Fisher's exact test. We approached this problem by calculating a so-called Dunn-Šidák correction; $\alpha = 1 - [1 - 0.05]^{1/\gamma}$; γ = times a set of data is used for a comparisons) (*Ludbrook 1998*). In all other cases, the probability of a Type I error < 0.05 was taken as the criterion of significance.

Results

One LEW rat was lost after blood sampling at day 42 and was not available for post mortem examination due to cannibalism. The final day 56 serum sample

from an SHR rat was lost during processing.

The rat strains differed in days 0, 9, and 21 background ELISA activities when evaluated by ANOVA (Table 1), for instance on day 0, BDIX, LEW and WKY rats versus SHR rats. *Post hoc* comparisons by the unpaired Student's *t* tests however did not yield significant differences in ELISA activities between the strains.

Exposure of rats to each other's enteric microflora for 3 weeks prior to *S. moniliformis* infection did not lead to the development of specific antibody activity to the bacterium.

Oral and intranasal infection at day 21 led to a significant antibody response in rats of all strains. At day 33 (day 12 *post infectionem* = p.i.) ELISA antibody levels significantly differed among the strains. WKY and SHR rats showed the highest antibody activities and the highest absolute seroconversion rates. BN and F344 rats showed the lowest antibody activities and absolute seroconversion rates. LEW and BD rats were intermediate both in antibody development and absolute seroconversion. BN, F344 and SHR rats had the highest background ELISA activity at day 0 and as a consequence a low relative seronversion.

All but 5 rats (a BN and four F344 rats excepted) were ELISA positive at day 33 (day 12 p.i.). The number of ELISA positive rats at day 33 differed among the strains.

Antibody development for the rat strains is also given in Fig. 1. All rats were ELISA positive at day 42 and day 56 and obviously no differences were observed any more in the number of seropositive rats after day 33 (Table 1).

The differences in ELISA antibody levels among the strains diminished after day 33 when day 42 and day 56 sera were tested at the 1:50 dilution (Fig. 1). As this observation suggested test limitations likely due to shortage of the secondary antibodies we retested the day 42 and day 56 sera at higher dilutions.

The pattern of differences among the strains found at day 33, now reappeared (Table 2 and Fig. 2). Generally highest antibody activities were measured in WKY and SHR rats; lowest activities in BN and F344 rats, and again LEW and BD rats were intermediate.

S. moniliformis was cultured or detected by PCR in pharyngeal samples from all but a few rats of the various strains (data not shown).

Discussion

There is a vast amount of literature indicating that inbred mouse and rat strains can significantly differ in susceptibility and antibody response to viral, bacterial and parasitic infections (*Kimman 2001*). Most studies involved non-rodent microorganisms mostly dosed to the animals in high numbers via unnatural routes (e.g. intraperitoneally).

There is a paucity of data on differences in disease susceptibility among rat strains to natural bacterial infections (*Kimman 2001*). Only four reports are on differences among rat strains in antibody response to rat pathogens, comprising *Corynebacterium kutscheri* (*Suzuki et al., 1998*), *Haemophilus* spp (*Boot et al., 2005*) and *Mycoplasma pulmonis* (*Davis et al., 1982*; *Simecka et al., 1989*).

Ours is the first study on antibody response to *S. moniliformis* in rat strains. The outcome suggests that the degree of antibody development to *S. moniliformis* is under genetic control. In studies in which outbred Wistar rats were exposed to *S. moniliformis*-infected counterparts in the same cage for 6 weeks, clear differences in seroconversion between cage mates were observed (*Boot et al., 1993*).

In mice, heritable variability in expression of streptobacillosis has been observed among inbred and hybrid mouse strains (*Wullenweber et al., 1990*). C57BL/6J mice developed much more pathology than BALB/cJ, C3H/He, DBA/2J and hybrid CB6F1 and B6D2F1 mice and the blacks were better antibody responders than DBA/2J and B6D2F1 mice. The genes and gene functions influencing disease susceptibilty and antibody response to infection may comprise innate immunity, lymphocyte function antigen processing and presentation, effector cell function and other mechanisms (Kimman 2001).

Overall WKY and SHR rats were high antibody responders to *S. moniliformis*, BN and F344 were low responders, and BD, LEW and SHR rats were intermediate.

C. kutscheri evoked a much better antibody response in LEW and SD rats than in BN and F344 (*Suzuki et al., 1998*). *Haemophilus* infection separated high responding BN rats from low reponding LEW rats (*Boot et al., 2005*). *M. pulmonis* infection resulted in higher antibody levels in LEW rats compared to F344 counterparts (*Davis et al., 1982*; *Simecka et al., 1989*). The data indicate that the response level of a rat strain to a given bacterium is, as yet, unpredictable. This indicates that outbred rats are preferably used in the serological monitoring of rat colonies for unwanted bacterial infections. The use of inbred rats, for example F344's, in monitoring colonies for *S. moniliformis* infection, would obviously increase the risk of false negative results of health monitoring.

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