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ORIGINAL ARTICLE

Establishment and Validation of a Mouse *Bacterial Vaginosis* Model

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SUMMARY

Background: ***Bacterial vaginosis*** **(BV) is a common vaginal infection without a reliable animal model. To establish a novel mouse BV model, we evaluated multiple parameters of various identified bacteria-infected mice, including *Staphylococcus aureus* (SA), *Escherichia coli* (EC), *Streptococcus agalactiae*, *β-Hemolytic streptococcus*, and *Gardnerella vaginalis* (GV).**

Methods: **Mature female KM mice were randomly allocated to a vehicle group (group A, without any treatment) and experimental groups. After vaginal secretions were harvested, experimental groups were divided into phosphate buffer solution group (PBS, group B), control group including SA, and EC with a 1:1 ratio (group C), SA, EC, and *Streptococcus agalactiae* with a 1:2:1 ratio group (group D), SA, EC, and *β-Hemolytic streptococcus* with a 1:2:1 ratio group (group E), and GV group (group F). The vaginal secretions of experimental mice were collected by flushing with 100 mL sterile PBS on days 2, 4, and 6. Vaginal secretions were examined by Gram staining, sialidase assay, ammonia test, and pH value measurement. IL-6 and IL-10 levels in mouse serum were detected by enzyme-linked immunosorbent assay. Hematoxylin-eosin staining and mouse cervicovaginal tissue histopathological scores were observed. The diagnostic test results were analyzed by logistic regression analysis and receiver operating characteristic curves. The Shapiro-Wilk analysis of variance, or rank-sum test, was used for normal distribution analysis. Pearson's correlation and chi-squared test determined the correlation and comparison data expressed as a percentage or frequency.**

Results: **There was less severe vaginal morphology in GV-infected mice compared to other bacteria-infected mice. The sialidase assay, the ammonia test, and the pH values of vaginal secretions showed significant differences between GV-infected and uninfected mice. Serum IL-6 and IL-10 levels and vaginal histological scoring increased in other bacteria-infected mice, but GV-infected mice showed only a mildly increasing trend of IL-10 levels and vaginal histological scoring compared to control mice.**

Conclusions: **GV-infected mice showed clinical features similar to human BV infection, including vaginal anatomical and pathological indices, and biochemical and immune parameters. Serum IL-10 level has potential for use in BV diagnosis.**

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Supplementary Data

Table S1. BV morbidity of each group (n = 10).

Group	BV morbidity (%)		p (α < 0.05)
	normal	BV	
A	10 (14.3)	0 (0)	0.000 **
B	9 (12.9)	1 (1.4)	0.000 **
C	2 (2.9)	8 (11.4)	0.000 ##**
D	1 (1.4)	9 (12.9)	0.000 ##**
E	1 (1.4)	9 (12.9)	0.000 ##**
F	0 (0)	10 (14.3)	0.000 ##**
Total	23 (32.9)	47 (67.1)	-

Compared with A - # p < 0.05, ## p < 0.01; among groups excluding A - * p< 0.05, ** p< 0.01.

Table S2. BV morbidity among each group (n = 10).

Group		Inspection group (Fisher's exact probabilities)					
		A	B	C	D	E	F
A	p	-	1.000	0.000	0.000	0.000	0.000
B	p	1.000	-	0.005	0.001	0.001	0.000
F	p	0.000	0.000	0.474	1.000	1.000	-

p < 0.05.

Table S3. Ammonia positive rate in each group (n = 10, %).

Group	First (2 d)		p (α < 0.05)	Second (4 d)		p (α < 0.05)	Third (6 d)		p (α < 0.05)
	-	+		-	+		-	+	
A	100	0	0.001 **	100	0	0.000 **	90	10	0.001 **
B	100	0	0.001 **	100	0	0.000 **	90	10	0.001 **
C	60	40	1.000	40	60	0.117	40	60	0.454
D	50	50	0.354	30	70	0.029 #*	20	80	0.027 #*
E	40	60	0.099	30	70	0.029 #*	20	80	0.027 #*
F	10	90	0.000 ##**	0	100	0.000 ##**	0	100	0.001 ##**

Compared with A - # p < 0.05, ## p < 0.01; among groups excluding A - * p < 0.05 ** p < 0.01.

Table S4. pH value of vaginal secretions (n = 10).

Group	First	p (α < 0.05)	Second	p (α < 0.05)	Third	p (α < 0.05)
A	6.39 ± 0.74	0.000 **	6.45±0.11	0.000 **	6.62 ± 0.23	0.000 **
B	7.03 ± 0.13	0.000 ##*	6.81±0.25	0.010 **	6.82 ± 0.18	0.000 **
C	6.72 ± 0.11	0.020 *	6.94±0.16	0.073	7.33 ± 0.22	0.004 ##
D	6.79 ± 0.16	0.024 #	7.22±0.15	0.000 ##	7.49 ± 0.14	0.000 ##**
E	6.84 ± 0.20	0.002 ##	7.20±0.16	0.000 ##	7.45 ± 0.16	0.000 ##**
F	6.89 ± 0.14	0.000 ##	7.29±0.15	0.000 ##**	7.46 ± 0.11	0.000 ##**

Compared with A - # p < 0.05, ## p < 0.01; among groups excluding A - * p < 0.05, ** p < 0.01.

Table S5. Serum levels of IL-6 and IL-10 (n = 10).

Group	IL-6 (pg/mL)	p (α < 0.05)	IL-10/(pg/mL)	p (α < 0.05)
A	13.09 ± 0.85	0.000 **	21.243 ± 0.83	0.000 **
B	14.75 ± 2.97	0.000 **	22.68 ± 1.64	0.000 **
C	26.87 ± 2.85	0.001 ##**	41.61 ± 0.91	0.001 ##*
D	29.22 ± 3.94	0.000 ##**	43.57 ± 1.76	0.000 ##**
E	31.01 ± 6.24	0.000 ##**	44.23 ± 1.88	0.000 ##**
F	19.89 ± 3.51	0.036 *	32.37 ± 2.79	0.019 *