Supplementary materials accompanying article: Early-life sickness may predispose Siberian hamsters to behavioral changes following alterations of the gut microbiome in adulthood, by Kristyn E. Sylvia, Jessica E. Deyoe, and Gregory E. Demas

Table 1. Effects of LPS treatment on the relative abundance of bacterial phyla in female and male adolescent microbiome. No values were significantly different across treatment groups.

Phylum	Statistics
Actinobacteria	$F_{1,39}$=2.479, p=0.267
Bacteroidetes	$F_{1,39}$=0.471, p=0.497
Cyanobacteria	$F_{1,39}$=2.304, p=0.267
Deferribacteres	$F_{1,39}$=3.124, p=0.267
Elusimicrobia	$F_{1,39}$=2.226, p=0.267
Euryarchaeota	$F_{1,39}$=1.266, p=0.408
Firmicutes	$F_{1,39}$=0.738, p=0.429
Fusobacteria	$F_{1,39}$=1.000, p=0.421
Proteobacteria	$F_{1,39}$=0.828, p=0.429
Saccharibacteria	$F_{1,39}$=2.845, p=0.267
Spirochaetae	$F_{1,39}$=1.190, p=0.408
Tenericutes	$F_{1,39}$=6.979, p=0.155
Verrucomicrobia	$F_{1,39}$=2.487, p=0.267

Table 2. Effects of LPS treatment on the relative abundance of bacterial families in female and male adolescents. An asterisk (*) indicates statistically significant differences between group means ($p<0.05$).

Family	Statistics
Alcaligenaceae	$F_{1,39}$=0.874, p=0.546
Anaeroplasmataceae	$F_{1,39}$=1.799, p=0.367
Bacteroidaceae	$F_{1,39}$=2.514, p=0.328
Bacteroidales RF16 group	$F_{1,39}$=0.438, p=0.667
Bacteroidales S24-7 group	$F_{1,39}$=0.395, p=0.675
Bifidobacteriaceae	$F_{1,39}$=1.857, p=0.367
Brachyspiraceae	**$F_{1,39}$=9.909, p=0.046***
Campylobacteraceae	$F_{1,39}$=3.725, p=0.275
Christensenellaceae	$F_{1,39}$=1.00, p=0.535
Clostridiales vadinBB60 group	$F_{1,39}$=0.359, p=0.679
Coriobacteriaceae	$F_{1,39}$=0.001, p=0.980
Corynebacteriaceae	$F_{1,39}$=4.922, p=0.265
Deferribacteraceae	$F_{1,39}$=3.124, p=0.282
Desulfovibrionaceae	$F_{1,39}$=0.075, p=0.913
Elusimicrobiaceae	$F_{1,39}$=2.226, p=0.344
Erysipelotrichaceae	$F_{1,39}$=0.018, p=0.980
Eubacteriaceae	$F_{1,39}$=0.003, p=0.980
Family XIII	$F_{1,39}$=2.502, p=0.328
Fusobacteriaceae	$F_{1,39}$=1.00, p=0.535
Helicobacteraceae	$F_{1,39}$=3.752, p=0.275
Lachnospiraceae	$F_{1,39}$=0.462, p=0.667
Lactobacillaceae	$F_{1,39}$=2.002, p=0.366

Methanosarcinaceae	$F_{1,39}$=1.266, p=0.500
Mitochondria	$F_{1,39}$=3.468, p=0.275
Mycoplasmataceae	$F_{1,39}$=0.501, p=0.667
Nitrosomonadaceae	$F_{1,39}$=1.00, p=0.535
Other 1	**$F_{1,39}$=14.175, p=0.012***
Other 2	$F_{1,39}$=2.845, p=0.307
Other 3	$F_{1,39}$=0.672, p=0.599
Oxalobacteraceae	$F_{1,39}$=0.671, p=0.599
Pasteurellaceae	$F_{1,39}$=3.625, p=0.275
Peptococcaceae	$F_{1,39}$=4.496, p=0.265
Porphyromonadaceae	**$F_{1,39}$=16.773, p=0.009***
Prevotellaceae	$F_{1,39}$=0.005, p=0.980
Rhodospirillaceae	$F_{1,39}$=0.156, p=0.831
Rickettsiales Incertae Sedis	$F_{1,39}$=0.001, p=0.980
Rikenellaceae	$F_{1,39}$=5.998, p=0.205
Ruminococcaceae	$F_{1,39}$=2.292, p=0.344
Spirochaetaceae	$F_{1,39}$=1.953, p=0.366
uncultured Firmicutes bacterium	$F_{1,39}$=0.898, p=0.546
uncultured rumen bacterium	$F_{1,39}$=4.381, p=0.265
uncultured Verrucomicrobia bacterium	$F_{1,39}$=0.038, p=0.958
Veillonellaceae	$F_{1,39}$=3.161, p=0.282

Figure 1. Principle Coordinates Analysis (PCoA) of the microbiome in adult females (black circles) and males (white circles). Gut microbial communities in adult females and males were not significantly different from each other ($F_{1,143}$=-70.557, p=0.974).

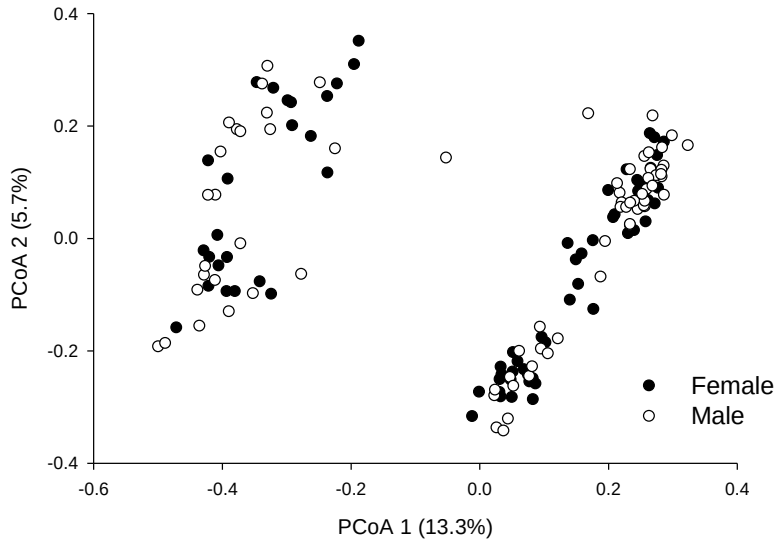

Figure 2. Box and whisker plot of Shannon-Wiener diversity across postnatal treatment groups (saline vs. LPS), adult treatment groups (water vs. antibiotics), and time (pre-treatment, treatment, and post-treatment) in female (a) and male (b) hamsters. Boxes represent the median and quartiles; whiskers represent the minimum and maximum. Outliers are represented as single open circles. Shannon diversity was significantly reduced after antibiotic treatment in both females and males when compared to water treatment, and it remained significantly reduced in antibiotic-treated animals after the seven-day recovery period. An asterisk (*) indicates statistically significant differences between groups ($p<0.05$).

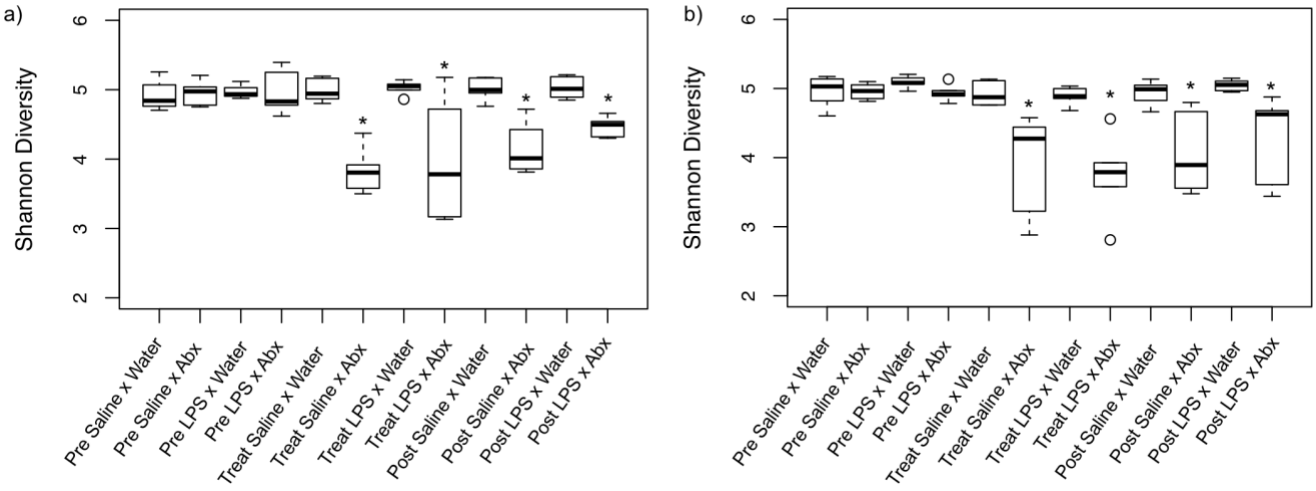

Table 3. Effects of adult antibiotic treatment on Bray-Curtis similarity in females.

	Pre-Water	Pre-Abx	Treat-Water	Treat-Abx	Post-Water
Pre-Abx	78.05%				
Treat-Water	78.35%	75.75%			
Treat-Abx	47.33%	48.70%	48.78%		
Post-Water	83.45%	80.39%	82.18%	48.35%	
Post-Abx	51.66%	52.66%	50.29%	65.11%	51.78%

Table 4. Effects of adult antibiotic treatment on Bray-Curtis similarity in males.

	Pre-Water	Pre-Abx	Treat-Water	Treat-Abx	Post-Water
Pre-Abx	78.48%				
Treat-Water	82.96%	76.45%			
Treat-Abx	40.62%	41.04%	41.84%		
Post-Water	82.41%	76.72%	82.95%	42.14%	
Post-Abx	51.52%	50.79%	52.50%	59.55%	52.54%

Table 5. Effects of adult antibiotic treatment on the relative abundance of bacterial phyla in the female microbiome. An asterisk (*) indicates statistically significant differences between group means ($p<0.05$).

Phyla	Variable	DF	F	p-value
Actinobacteria	Adult Treatment x Timepoint	2, 42	2.098	0.465
Actinobacteria	Postnatal Treatment x Timepoint	2, 42	1.850	0.510
Actinobacteria	Adult Treatment	1, 20	1.107	0.712
Actinobacteria	Postnatal Treatment x Adult Treatment	1, 20	0.812	0.775
Actinobacteria	Postnatal Treatment	1, 20	0.435	0.866
Actinobacteria	Timepoint	3, 46	0.439	0.935
Actinobacteria	Postnatal Treatment x Adult Treatment x Timepoint	2, 42	0.245	0.954
Bacteroidetes	Postnatal Treatment	1, 59	5.390	0.245
Bacteroidetes	Timepoint	3, 59	2.988	0.272
Bacteroidetes	Adult Treatment	1, 59	2.631	0.441
Bacteroidetes	Postnatal Treatment x Adult Treatment	1, 59	2.027	0.497
Bacteroidetes	Postnatal Treatment x Timepoint	2, 59	0.664	0.866
Bacteroidetes	Adult Treatment x Timepoint	2, 59	0.428	0.894
Bacteroidetes	Postnatal Treatment x Adult Treatment x Timepoint	2, 59	0.076	0.985
Cyanobacteria	**Adult Treatment**	**1, 20**	**20.298**	**0.009***
Cyanobacteria	Timepoint	3, 44	2.988	0.272
Cyanobacteria	Adult Treatment x Timepoint	2, 40	2.440	0.421
Cyanobacteria	Postnatal Treatment x Timepoint	2, 40	1.226	0.712
Cyanobacteria	Postnatal Treatment	1, 20	0.928	0.747
Cyanobacteria	Postnatal Treatment x Adult Treatment	1, 20	0.032	0.963
Cyanobacteria	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.010	0.996
Deferribacteres	Postnatal Treatment	1, 20	3.176	0.414
Deferribacteres	Adult Treatment x Timepoint	2, 41	1.449	0.659
Deferribacteres	Postnatal Treatment x Adult Treatment x Timepoint	2, 41	1.256	0.712
Deferribacteres	Adult Treatment	1, 20	0.380	0.866
Deferribacteres	Postnatal Treatment x Adult Treatment	1, 20	0.205	0.894
Deferribacteres	Postnatal Treatment x Timepoint	2, 41	0.016	0.996
Deferribacteres	Timepoint	3, 45	0.022	0.996
Elusimicrobia	Postnatal Treatment x Adult Treatment	1, 20	2.371	0.465
Elusimicrobia	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	1.733	0.550
Elusimicrobia	Timepoint	3, 45	1.323	0.709
Elusimicrobia	Postnatal Treatment	1, 20	0.102	0.935
Elusimicrobia	Postnatal Treatment x Timepoint	2, 40	0.280	0.935
Elusimicrobia	Adult Treatment x Timepoint	2, 40	0.227	0.957
Elusimicrobia	Adult Treatment	1, 20	0.022	0.963
Euryarchaeota	Adult Treatment	1, 60	5.750	0.245
Euryarchaeota	Postnatal Treatment	1, 60	3.415	0.344
Euryarchaeota	Timepoint	3, 60	1.873	0.465
Euryarchaeota	Postnatal Treatment x Timepoint	2, 60	0.839	0.841
Euryarchaeota	Postnatal Treatment x Adult Treatment x Timepoint	2, 60	0.581	0.866
Euryarchaeota	Postnatal Treatment x Adult Treatment	1, 60	0.314	0.866
Euryarchaeota	Adult Treatment x Timepoint	2, 60	0.447	0.894
Firmicutes	Postnatal Treatment	1, 59	5.223	0.245
Firmicutes	Timepoint	3, 59	2.607	0.315
Firmicutes	Postnatal Treatment x Adult Treatment	1, 59	2.433	0.465

Firmicutes	Adult Treatment	1, 59	0.995	0.726
Firmicutes	Postnatal Treatment x Timepoint	2, 59	0.785	0.841
Firmicutes	Adult Treatment x Timepoint	2, 59	0.312	0.935
Firmicutes	Postnatal Treatment x Adult Treatment x Timepoint	2, 59	0.100	0.974
Proteobacteria	**Adult Treatment**	**1, 20**	**32.719**	**0.001***
Proteobacteria	**Timepoint**	**3, 45**	**6.016**	**0.033***
Proteobacteria	Adult Treatment x Timepoint	2, 40	3.253	0.275
Proteobacteria	Postnatal Treatment	1, 20	3.095	0.414
Proteobacteria	Postnatal Treatment x Adult Treatment	1, 20	0.261	0.894
Proteobacteria	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.177	0.963
Proteobacteria	Postnatal Treatment x Timepoint	2, 40	0.128	0.963
Saccharibacteria	Adult Treatment x Timepoint	2, 40	5.710	0.111
Saccharibacteria	Timepoint	3, 42	3.400	0.245
Saccharibacteria	Adult Treatment	1, 20	5.060	0.272
Saccharibacteria	Postnatal Treatment x Adult Treatment	1, 20	0.370	0.866
Saccharibacteria	Postnatal Treatment	1, 20	0.323	0.866
Saccharibacteria	Postnatal Treatment x Timepoint	2, 40	0.559	0.866
Saccharibacteria	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.018	0.996
Spirochaetae	Adult Treatment	1, 20	4.549	0.273
Spirochaetae	Timepoint	3, 44	1.616	0.558
Spirochaetae	Adult Treatment x Timepoint	2, 39	0.845	0.841
Spirochaetae	Postnatal Treatment	1, 20	0.212	0.894
Spirochaetae	Postnatal Treatment x Adult Treatment x Timepoint	2, 39	0.382	0.914
Spirochaetae	Postnatal Treatment x Timepoint	2, 39	0.138	0.963
Spirochaetae	Postnatal Treatment x Adult Treatment	1, 20	0.000	0.996
Tenericutes	**Adult Treatment**	**1, 20**	**13.515**	**0.033***
Tenericutes	Adult Treatment x Timepoint	2, 40	3.433	0.272
Tenericutes	Postnatal Treatment	1, 20	2.390	0.465
Tenericutes	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	1.145	0.726
Tenericutes	Timepoint	3, 44	1.082	0.770
Tenericutes	Postnatal Treatment x Timepoint	2, 40	0.804	0.841
Tenericutes	Postnatal Treatment x Adult Treatment	1, 20	0.050	0.963
Verrucomicrobia	Postnatal Treatment	1, 4	1.809	0.659
Verrucomicrobia	Adult Treatment	1, 4	0.719	0.841
Verrucomicrobia	Postnatal Treatment x Adult Treatment x Timepoint	2, 4	0.877	0.849
Verrucomicrobia	Timepoint	3, 4	0.986	0.849
Verrucomicrobia	Postnatal Treatment x Timepoint	2, 4	0.463	0.894
Verrucomicrobia	Adult Treatment x Timepoint	2, 4	0.303	0.935
Verrucomicrobia	Postnatal Treatment x Adult Treatment	1, 4	0.032	0.963

Table 6. Effects of adult antibiotic treatment on the relative abundance of bacterial phyla in the male microbiome. An asterisk (*) indicates statistically significant differences between group means ($p<0.05$).

Phylum	Variable	DF	F	p-value
Actinobacteria	Postnatal Treatment	1, 20	7.392	0.101
Actinobacteria	Postnatal Treatment x Timepoint	2, 40	1.368	0.785
Actinobacteria	Adult Treatment	1, 20	0.913	0.796
Actinobacteria	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.823	0.845
Actinobacteria	Timepoint	2, 40	0.232	0.962
Actinobacteria	Adult Treatment x Timepoint	2, 40	0.224	0.962
Actinobacteria	Postnatal Treatment x Adult Treatment	1, 20	0.047	0.962
Bacteroidetes	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	1.710	0.775
Bacteroidetes	Adult Treatment	1, 20	1.224	0.785
Bacteroidetes	Postnatal Treatment x Adult Treatment	1, 20	0.466	0.845
Bacteroidetes	Adult Treatment x Timepoint	2, 40	0.152	0.962
Bacteroidetes	Timepoint	2, 40	0.128	0.962
Bacteroidetes	Postnatal Treatment x Timepoint	2, 40	0.110	0.962
Bacteroidetes	Postnatal Treatment	1, 20	0.007	0.962
Cyanobacteria	**Timepoint**	**2, 40**	**11.729**	**0.003***
Cyanobacteria	**Adult Treatment**	**1, 20**	**19.325**	**0.004***
Cyanobacteria	Postnatal Treatment	1, 20	5.111	0.227
Cyanobacteria	Adult Treatment x Timepoint	2, 40	2.500	0.498
Cyanobacteria	Postnatal Treatment x Adult Treatment	1, 20	1.466	0.785
Cyanobacteria	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.522	0.961
Cyanobacteria	Postnatal Treatment x Timepoint	2, 40	0.301	0.962
Deferribacteres	Adult Treatment	1, 20	1.235	0.785
Deferribacteres	Postnatal Treatment x Adult Treatment	1, 20	1.130	0.785
Deferribacteres	Postnatal Treatment	1, 20	0.877	0.796
Deferribacteres	Adult Treatment x Timepoint	2, 40	1.047	0.796
Deferribacteres	Timepoint	2, 40	0.931	0.821
Deferribacteres	Postnatal Treatment x Timepoint	2, 40	0.868	0.836
Deferribacteres	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.754	0.845
Elusimicrobia	Timepoint	2, 40	4.638	0.108
Elusimicrobia	Adult Treatment	1, 20	2.478	0.612
Elusimicrobia	Postnatal Treatment x Timepoint	2, 40	0.424	0.961
Elusimicrobia	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.409	0.961
Elusimicrobia	Postnatal Treatment	1, 20	0.048	0.962
Elusimicrobia	Postnatal Treatment x Adult Treatment	1, 20	0.034	0.962
Elusimicrobia	Adult Treatment x Timepoint	2, 40	0.103	0.962
Euryarchaeota	Adult Treatment x Timepoint	2, 40	2.076	0.613
Euryarchaeota	Postnatal Treatment	1, 20	1.692	0.785
Euryarchaeota	Adult Treatment	1, 20	1.230	0.785
Euryarchaeota	Postnatal Treatment x Adult Treatment	1, 20	0.275	0.961
Euryarchaeota	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.482	0.961
Euryarchaeota	Timepoint	2, 40	0.397	0.961
Euryarchaeota	Postnatal Treatment x Timepoint	2, 40	0.034	0.966
Firmicutes	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	1.799	0.750

Firmicutes	Adult Treatment	1, 20	0.507	0.845
Firmicutes	Postnatal Treatment x Adult Treatment	1, 20	0.471	0.845
Firmicutes	Timepoint	2, 40	0.465	0.961
Firmicutes	Adult Treatment x Timepoint	2, 40	0.457	0.961
Firmicutes	Postnatal Treatment x Timepoint	2, 40	0.113	0.962
Firmicutes	Postnatal Treatment	1, 20	0.004	0.962
Proteobacteria	**Adult Treatment**	**1, 20**	**19.775**	**0.004***
Proteobacteria	**Timepoint**	**2, 40**	**9.974**	**0.004***
Proteobacteria	Adult Treatment x Timepoint	2, 40	5.342	0.074
Proteobacteria	Postnatal Treatment x Adult Treatment	1, 20	0.057	0.962
Proteobacteria	Postnatal Treatment	1, 20	0.044	0.962
Proteobacteria	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.095	0.962
Proteobacteria	Postnatal Treatment x Timepoint	2, 40	0.059	0.962
Saccharibacteria	**Timepoint**	**2, 40**	**16.306**	**0.003***
Saccharibacteria	**Adult Treatment x Timepoint**	**2, 40**	**9.299**	**0.006***
Saccharibacteria	Adult Treatment	1, 20	2.580	0.612
Saccharibacteria	Postnatal Treatment x Adult Treatment	1, 20	0.191	0.961
Saccharibacteria	Postnatal Treatment	1, 20	0.070	0.962
Saccharibacteria	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.175	0.962
Saccharibacteria	Postnatal Treatment x Timepoint	2, 40	0.082	0.962
Spirochaetae	Postnatal Treatment x Adult Treatment	1, 20	1.549	0.785
Spirochaetae	Adult Treatment	1, 20	1.340	0.785
Spirochaetae	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	1.364	0.785
Spirochaetae	Postnatal Treatment	1, 20	1.092	0.785
Spirochaetae	Postnatal Treatment x Timepoint	2, 40	0.983	0.821
Spirochaetae	Adult Treatment x Timepoint	2, 40	0.307	0.962
Spirochaetae	Timepoint	2, 40	0.273	0.962
Tenericutes	**Adult Treatment x Timepoint**	**2, 40**	**15.333**	**0.003***
Tenericutes	**Timepoint**	**2, 40**	**7.109**	**0.024***
Tenericutes	**Adult Treatment**	**1, 20**	**10.025**	**0.046***
Tenericutes	Postnatal Treatment x Timepoint	2, 40	1.091	0.796
Tenericutes	Postnatal Treatment x Adult Treatment	1, 20	0.925	0.796
Tenericutes	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.354	0.962
Tenericutes	Postnatal Treatment	1, 20	0.113	0.962
Verrucomicrobia	Timepoint	2, 40	3.219	0.303
Verrucomicrobia	Adult Treatment	1, 20	3.185	0.498
Verrucomicrobia	Postnatal Treatment x Timepoint	2, 40	1.224	0.785
Verrucomicrobia	Postnatal Treatment x Adult Treatment x Timepoint	2, 40	0.933	0.821
Verrucomicrobia	Postnatal Treatment	1, 20	0.707	0.821
Verrucomicrobia	Adult Treatment x Timepoint	2, 40	0.772	0.845
Verrucomicrobia	Postnatal Treatment x Adult Treatment	1, 20	0.480	0.845

Table 7. Significant effects of treatment on the relative abundance of bacterial families in the female microbiome. All values represent statistically significant differences between group means ($p<0.05$).

Family	Variable	DF	F	p-value
Bacteroidaceae	Adult Treatment	1, 20	12.961	0.027
Bacteroidales S24-7 group	Adult Treatment	1, 59	17.371	0.005
Bacteroidales S24-7 group	Timepoint	3, 59	5.625	0.027
Brachyspiraceae	Adult Treatment	1, 17	11.995	0.037
Clostridiales vadinBB60 group	Adult Treatment x Timepoint	2, 39	10.021	0.011
Clostridiales vadinBB60 group	Adult Treatment	1, 20	15.154	0.019
Clostridiales vadinBB60 group	Timepoint	3, 42	5.410	0.038
Corynebacteriaceae	Adult Treatment	1, 20	15.081	0.019
Corynebacteriaceae	Postnatal Treatment x Timepoint	2, 39	7.765	0.023
Desulfovibrionaceae	Adult Treatment	1, 59	18.894	0.005
Desulfovibrionaceae	Timepoint	3, 59	6.904	0.014
Family XIII	Timepoint	3, 55	6.613	0.017
Family XIII	Adult Treatment	1, 17	15.292	0.021
Helicobacteraceae	Adult Treatment	1, 20	18.634	0.011
Helicobacteraceae	Adult Treatment x Timepoint	2, 40	7.098	0.032
Mycoplasmataceae	Adult Treatment	1, 20	12.115	0.032
Other	Adult Treatment	1, 20	23.926	0.005
Oxalobacteraceae	Adult Treatment	1, 111	19.644	0.005
Peptococcaceae	Timepoint	3, 61	4.992	0.042
Porphyromonadaceae	Adult Treatment	1, 20	24.295	0.005
Porphyromonadaceae	Adult Treatment x Timepoint	2, 40	8.259	0.019
Prevotellaceae	Adult Treatment	1, 20	15.138	0.019
Rikenellaceae	Adult Treatment x Timepoint	2, 39	11.845	0.005
Rikenellaceae	Adult Treatment	1, 20	21.202	0.008
Ruminococcaceae	Postnatal Treatment	1, 59	9.274	0.041
uncultured bacterium	Adult Treatment	1, 20	13.903	0.023
uncultured rumen bacterium	Adult Treatment	1, 20	16.581	0.016

Table 8. Significant effects of treatment on the relative abundance of bacterial families in the male microbiome. All values represent statistically significant differences between group means ($p<0.05$).

Family	Variable	DF	F	p-value
Bacteroidales RF16	Timepoint	2, 40	11.104	0.002
Bacteroidales RF16	Adult Treatment	1, 20	9.682	0.049
Brachyspiraceae	Adult Treatment x Timepoint	2, 40	10.300	0.003
Campylobacteraceae	Timepoint	2, 40	8.480	0.010
Clostridiales vadinBB60 group	Adult Treatment	1, 20	21.126	0.003
Clostridiales vadinBB60	Timepoint	2, 40	10.842	0.003
Clostridiales vadinBB60	Adult Treatment x Timepoint	2, 40	10.694	0.003
Family XIII	Adult Treatment	1, 20	39.041	0.002
Helicobacteraceae	Timepoint	2, 40	14.753	0.002
Helicobacteraceae	Adult Treatment	1, 20	21.367	0.003
Helicobacteraceae	Adult Treatment x Timepoint	2, 40	8.439	0.010
Mycoplasmataceae	Timepoint	2, 40	13.360	0.002
Mycoplasmataceae	Adult Treatment x Timepoint	2, 40	7.786	0.015
Mycoplasmataceae	Adult Treatment	1, 20	10.884	0.033
Other 1	Timepoint	2, 40	11.512	0.002

Other 1	Adult Treatment x Timepoint	2, 40	7.134	0.022
Other 1	Adult Treatment	1, 20	11.162	0.031
Oxalobacteraceae	Adult Treatment	1, 20	15.307	0.010
Peptococcaceae	Adult Treatment	1, 20	20.398	0.003
Porphyromonadaceae	Timepoint	2, 40	21.441	0.002
Porphyromonadaceae	Adult Treatment x Timepoint	2, 40	16.462	0.002
Porphyromonadaceae	Adult Treatment	1, 20	20.163	0.003
Prevotellaceae	Timepoint	2, 40	16.517	0.002
Prevotellaceae	Adult Treatment x Timepoint	2, 40	11.847	0.002
Prevotellaceae	Adult Treatment	1, 20	19.800	0.003
Rhodospirillaceae	Timepoint	2, 40	16.556	0.002
Rikenellaceae	Timepoint	2, 40	21.260	0.002
Rikenellaceae	Adult Treatment x Timepoint	2, 40	7.312	0.021
Other 2	Adult Treatment	1, 20	21.794	0.002
Other 2	Timepoint	2, 40	10.216	0.004
Uncultured rumen bacterium	Adult Treatment	1, 20	11.249	0.031
Other 3	Timepoint	2, 40	16.306	0.002
Other 3	Adult Treatment x Timepoint	2, 40	9.299	0.006

Table 9. Means ± SEM of female behavior across treatment groups and time. No values were significantly different from one another ($p>0.05$). To determine effects of treatment on behavior, we included litter as a random effect.

	Day 71				Day 78				Day 85			
Behavior	**Saline x Water**	**Saline x Abx**	**LPS x Water**	**LPS x Abx**	**Saline x Water**	**Saline x Abx**	**LPS x Water**	**LPS x Abx**	**Saline x Water**	**Saline x Abx**	**LPS x Water**	**LPS x Abx**
Duration of anogenital investigation	26.33 ± 4.78	20.29 ± 6.39	16.43 ± 3.68	18.62 ± 6.58	18.53 ± 3.99	15.27 ± 5.31	18.00 ± 5.65	11.62 ± 3.13	9.99 ± 2.90	12.41 ± 4.08	14.53 ± 3.35	17.48 ± 5.13
Frequency of anogenital investigation	12.25 ± 2.84	8.56 ± 2.76	8.83 ± 2.68	8.17 ± 1.97	9.75 ± 1.93	9.56 ± 2.82	11.00 ± 3.26	10.67 ± 2.35	7.00 ± 1.72	10.44 ± 2.87	9.50 ± 1.78	11.00 ± 2.72
Duration of nose-to-nose investigation	24.25 ± 5.71	14.17 ± 3.45	17.47 ± 7.78	12.43 ± 3.11	14.79 ± 3.69	17.46 ± 4.45	13.52 ± 4.66	9.78 ± 1.91	12.36 ± 3.57	11.64 ± 3.76	6.13 ± 4.05	6.18 ± 1.47
Frequency of nose-to-nose investigation	15.50 ± 2.15	12.11 ± 2.44	13.50 ± 3.98	12.67 ± 2.08	19.38 ± 2.85	18.44 ± 3.01	13.50 ± 2.39	15.33 ± 2.47	17.75 ± 3.23	15.78 ± 3.09	11.50 ± 5.84	11.83 ± 2.36
Number of Attacks	3.63 ± 1.39	6.00 ± 2.57	3.17 ± 1.62	2.50 ± 1.15	1.38 ± 0.63	2.78 ± 1.02	1.83 ± 0.91	2.50 ± 0.62	1.00 ± 0.42	1.56 ± 0.56	2.67 ± 0.99	1.83 ± 0.65
Duration of Attacks	3.59 ± 1.36	3.81 ± 1.37	2.20 ± 1.02	1.77 ± 0.93	2.01 ± 0.84	2.26 ± 0.88	1.58 ± 0.65	2.53 ± 0.71	1.51 ± 0.71	1.67 ± 0.56	2.53 ± 1.26	1.75 ± 0.62
Number of Chases	0.13 ± 0.13	1.11 ± 0.59	0.00 ± 0.00	0.17 ± 0.17	0.25 ± 0.25	0.67 ± 0.44	0.00 ± 0.00	0.17 ± 0.17	0.00 ± 0.00	0.11 ± 0.11	0.17 ± 0.17	0.00 ± 0.00
Duration of Chases	0.05 ± 0.05	0.79 ± 0.42	0.00 ± 0.00	0.08 ± 0.08	0.19 ± 0.19	0.41 ± 0.31	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00	0.10 ± 0.10	0.08 ± 0.08	0.00 ± 0.00
Latency to First Attack	79.13 ± 35.93	62.11 ± 19.38	121.33 ± 43.23	99.33 ± 28.55	53.50 ± 23.95	46.56 ± 25.40	31.67 ± 11.59	82.83 ± 24.91	32.63 ± 16.34	61.89 ± 27.38	112.17 ± 37.96	57.50 ± 31.23
Duration of Grooming	13.71 ± 4.82	13.89 ± 3.08	8.90 ± 2.15	14.22 ± 6.82	7.85 ± 2.48	16.67 ± 5.17	10.57 ± 3.77	11.30 ± 6.25	6.65 ± 2.78	11.20 ± 4.01	4.60 ± 2.11	7.62 ± 2.97
Frequency of Grooming	7.63 ± 2.37	6.22 ± 1.14	5.17 ± 1.05	8.17 ± 2.36	4.50 ± 1.57	7.33 ± 1.71	6.17 ± 2.14	5.00 ± 1.69	3.38 ± 0.82	4.33 ± 1.17	2.67 ± 1.31	5.33 ± 1.26

Table 10. Means ± SEM of male behavior across treatment groups and time. An asterisk (*) indicates statistically significant effect of treatment with the random effect of litter included ($p<0.05$).

	Day 71				Day 78				Day 85			
Behavior	**Saline x Water**	**Saline x Abx**	**LPS x Water**	**LPS x Abx**	**Saline x Water**	**Saline x Abx**	**LPS x Water**	**LPS x Abx**	**Saline x Water**	**Saline x Abx**	**LPS x Water**	**LPS x Abx**
Duration of anogenital investigation	22.79 ± 6.03	18.29 ± 5.48	19.26 ± 10.65	14.11 ± 4.12	13.27 ± 6.19	8.13 ± 3.86	15.83 ± 8.07	9.86 ± 4.22	12.46 ± 4.05	10.79 ± 4.48	16.44 ± 10.91	13.70 ± 4.09
Frequency of anogenital investigation	9.86 ± 2.43	7.71 ± 2.01	7.00 ± 2.69	7.38 ± 1.57	9.14 ± 4.06	5.14 ± 2.25	11.29 ± 5.19	7.25 ± 2.84	10.71 ± 2.83	8.29 ± 3.16	9.14 ± 4.76	12.00 ± 3.69
Duration of nose-to-nose investigation	39.01 ± 14.94	31.14 ± 13.52	12.49 ± 5.29	30.51 ± 7.04	14.49 ± 4.37	***8.90 ± 4.65**	12.16 ± 6.21	***12.45 ± 3.67**	25.04 ± 11.78	***6.56 ± 2.75**	6.83 ± 3.11	***9.83 ± 2.23**
Frequency of nose-to-nose investigation	21.57 ± 5.81	15.14 ± 6.32	8.43 ± 1.67	16.50 ± 3.15	18.00 ± 4.13	10.29 ± 5.53	12.43 ± 4.53	14.13 ± 3.44	23.14 ± 8.10	11.43 ± 4.13	12.71 ± 4.57	15.50 ± 3.18
Number of Attacks	6.29 ± 1.71	13.29 ± 3.08	8.71 ± 2.91	5.50 ± 1.40	2.86 ± 0.80	9.00 ± 3.45	3.71 ± 0.92	4.50 ± 2.31	2.29 ± 0.68	6.43 ± 1.34	4.86 ± 1.45	4.00 ± 1.04
Duration of Attacks	9.77 ± 3.61	24.29 ± 9.75	17.97 ± 7.88	14.65 ± 4.47	3.41 ± 1.36	16.96 ± 11.44	4.33 ± 1.39	6.51 ± 3.22	2.11 ± 0.83	8.30 ± 3.11	5.30 ± 1.94	4.64 ± 1.26
Number of Chases	0.14 ± 0.14	4.14 ± 2.55	0.71 ± 0.47	0.38 ± 0.26	0.00 ± 0.00	0.86 ± 0.55	0.00 ± 0.00	0.75 ± 0.75	0.00 ± 0.00	0.57 ± 0.57	0.00 ± 0.00	0.25 ± 0.25
Duration of Chases	0.07 ± 0.07	4.17 ± 2.57	0.50 ± 0.32	0.21 ± 0.15	0.00 ± 0.00	0.46 ± 0.30	0.00 ± 0.00	0.55 ± 0.55	0.00 ± 0.00	0.44 ± 0.44	0.00 ± 0.00	0.11 ± 0.11
Latency to First Attack	21.71 ± 6.30	65.86 ± 20.66	120.57 ± 39.36	62.00 ± 27.02	34.71 ± 13.37	48.71 ± 13.57	53.86 ± 24.14	48.75 ± 27.32	113.14 ± 39.85	46.86 ± 18.70	56.29 ± 31.98	64.63 ± 21.18
Duration of Grooming	7.79 ± 2.89	5.49 ± 1.63	3.94 ± 1.96	6.21 ± 0.94	7.09 ± 2.65	17.94 ± 10.78	5.01 ± 0.98	12.13 ± 2.21	5.74 ± 1.13	5.60 ± 2.57	4.21 ± 2.02	9.88 ± 1.94
Frequency of Grooming	5.43 ± 2.21	2.86 ± 0.77	3.57 ± 1.46	3.50 ± 0.71	5.29 ± 1.43	3.57 ± 1.21	4.00 ± 0.98	***9.00 ± 2.22**	4.14 ± 1.14	3.14 ± 1.16	2.43 ± 0.90	***7.13 ± 1.17**