Supplemental Material

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TITLE:

Protection generated by prior exposure to pathogens depends on both priming and challenge dose

AUTHORS:

Chava L. Weitzman*, Guadalupe Ceja, Ariel E. Leon, Dana M. Hawley

* weitzman.chava@gmail.com

Summary:

Results are provided for analyses that include data on all birds, including those inoculated with the sham-MG treatment (Table S1) and analyses excluding birds given topical ocular antibiotic treatment prior to the priming MG exposure (Table S2). Fig S1 provides results on pathology and MG load after the priming MG treatment. Fig S2 provides anti-MG ELISA absorbance data during the secondary challenge. Fig S3 provides data on MG load in birds that were successfully infected by the secondary challenge.

Table S1. Analyses including secondary sham birds provided similar results to analyses without the sham birds (see *Main Text*). RID = reinfection day. Bold denotes significant results.

Probability of reinfection	**Estimate ± SEM**	**z value**	***P***
(Intercept)	5.620 ± 1.969	2.854	**0.004**
priming	–1.525 ± 0.538	–2.835	**0.005**
secondary – sham	–11.196 ± 1626.3	–0.007	1
secondary – low	–5.835 ± 2.000	–2.918	**0.004**
priming*secondary – sham	1.525 ± 441.2	0.003	1
priming*secondary – low	1.373 ± 0.556	2.469	**0.01**
Probability of reinfection			
(Intercept)	2.981 ± 0.833	3.581	**0.0003**
RID 0 ELISA	–30.277 ± 9.972	–3.036	**0.002**
secondary – sham	–6.498 ± 352.5	–0.018	1
secondary – low	–1.298 ± 0.422	–3.076	**0.002**
Eye score during reinfection			
(Intercept)	0.301 ± 0.336	0.895	0.4
priming^2	–0.169 ± 0.044	–3.888	**0.0001**
secondary – sham	–47.3 ± 19860	–0.002	1
secondary – low	–3.030 ± 0.617	–4.910	**<0.0001**
priming^2*secondary – sham	2.336 ± 990.9	0.002	1
priming^2*secondary – low	0.068 ± 0.092	0.744	0.5
MG load during reinfection			
(Intercept)	2.256 ± 0.247	9.135	**<0.0001**
Temporal group – 2	–0.315 ± 0.185	–1.700	0.089
RID	–0.031 ± 0.017	–1.827	0.068
priming^2	–0.067 ± 0.023	–2.875	**0.004**
secondary - sham	–4.469 ± 1.150	–3.886	**0.0001**
secondary - low	–2.630 ± 0.353	–7.445	**<0.0001**
RID*priming^2	–0.004 ± 0.002	–1.710	0.087
RID*secondary – sham	0.253 ± 0.090	2.828	**0.005**
RID*secondary – low	0.094 ± 0.026	3.578	**0.0003**
priming^2*secondary – sham	0.108 ± 0.075	1.440	0.15
priming^2*secondary – low	0.093 ± 0.027	3.477	**0.0005**

Table S2. Analyses excluding birds given ocular antibiotics prior to the priming dose provided similar results to analyses without the sham birds (see *Main Text*) and analyses including all birds (see Table S1). RID = reinfection day. Bold denotes significant results.

Probability of reinfection	**Estimate ± SEM**	**z value**	***P***
(Intercept)	5.373 ± 2.046	2.628	**0.009**
priming	–1.464 ± 0.554	–2.643	**0.008**
secondary – low	–5.238 ± 2.089	–2.507	**0.012**
priming*secondary – low	1.152 ± 0.581	1.983	**0.047**
Probability of reinfection			
(Intercept)	2.561 ± 0.870	2.945	**0.003**
RID 0 ELISA	–26.444 ± 10.030	–2.637	**0.008**
secondary – low	–1.191 ± 0.447	–2.665	**0.008**
Eye score during reinfection			
(Intercept)	0.462 ± 0.433	1.068	0.3
priming^2	–0.193 ± 0.051	–3.758	**0.0002**
secondary – low	–3.021 ± 0.757	–3.992	**<0.0001**
priming^2*secondary – low	0.035 ± 0.124	0.279	0.8
MG load during reinfection			
(Intercept)	2.114 ± 0.273	7.745	**<0.0001**
RID	–0.039 ± 0.022	–1.800	0.072
priming^2	–0.088 ± 0.028	–3.106	**0.002**
secondary – low	–2.899 ± 0.461	–6.292	**<0.0001**
RID*priming^2	–0.0005 ± 0.003	–0.176	0.9
RID*secondary – low	0.144 ± 0.038	3.805	**0.0001**
priming^2*secondary – low	0.158 ± 0.046	3.416	**0.0006**
RID*priming^2*secondary – low	-0.010 ± 0.005	–2.206	**0.027**

Fig S1. Infection results after priming inoculation. (A) Total eye score as sum of all eye scores over five sampling days before re-inoculation (day 3 to day 27 post-priming inoculation). Values are averages plus standard deviations. (B) Probability of infection, with successful infection delineated by any MGC2 qPCR value greater than $\log_{10}$ 3.1 copies prior to the reinfection challenge. qPCRs were conducted on samples from post-priming inoculation days 3 and 13. Data represent a subset of the total birds used in a separate experiment (Weitzman et al. in review).

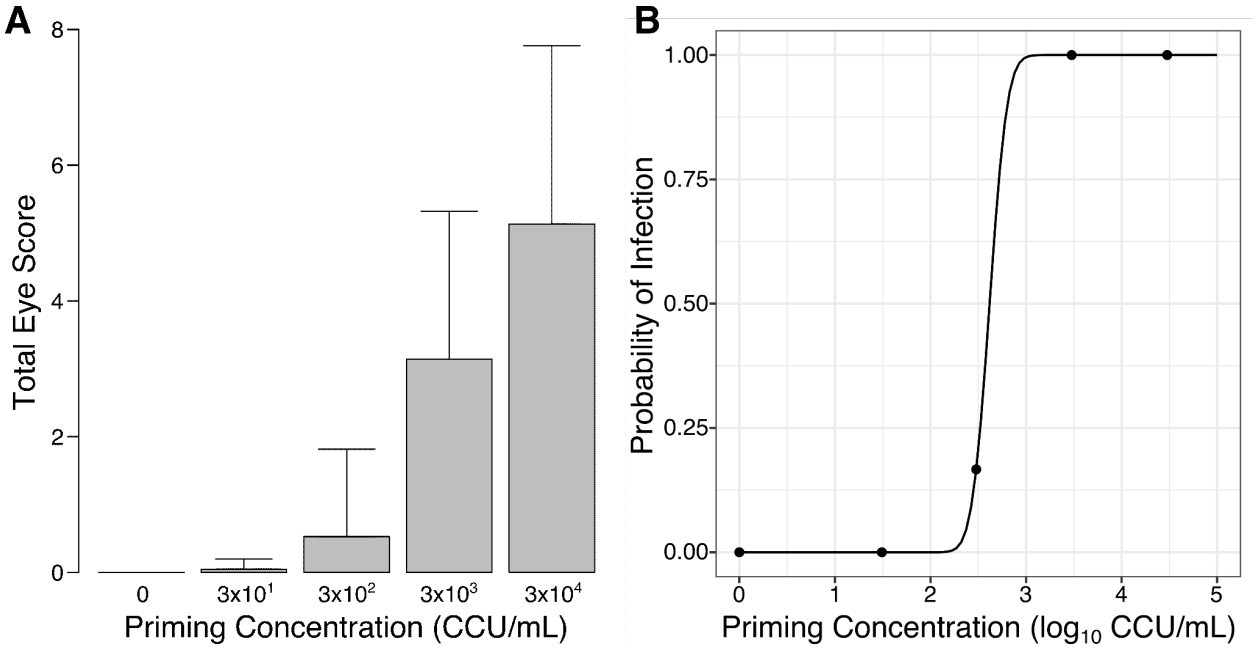


Fig S2. *Mycoplasma gallisepticum* (MG)-specific antibody levels 14 days after reinfection (RID 14) differed by secondary challenge dose ($F_{2,68} = 8.55$, $p = 0.0005$) and the interaction between priming and challenge dose ($F_{2,68} = 4.00$, $p = 0.02$). Data are color-coded by challenge dose treatment. Dotted line at 0.0671 absorbance represents the conservative value used to determine seroconversion (see *Methods*). High and low dose challenge treatments, n = 4–8. Sham challenge treatment, n = 2–3.

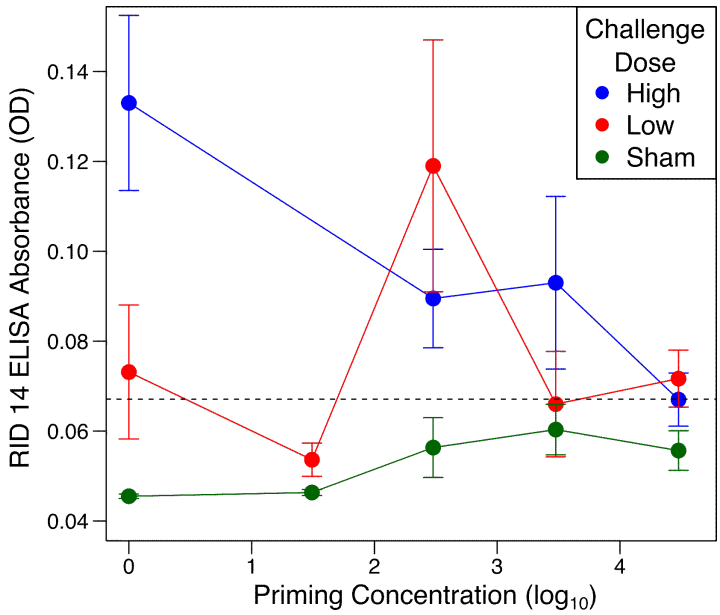
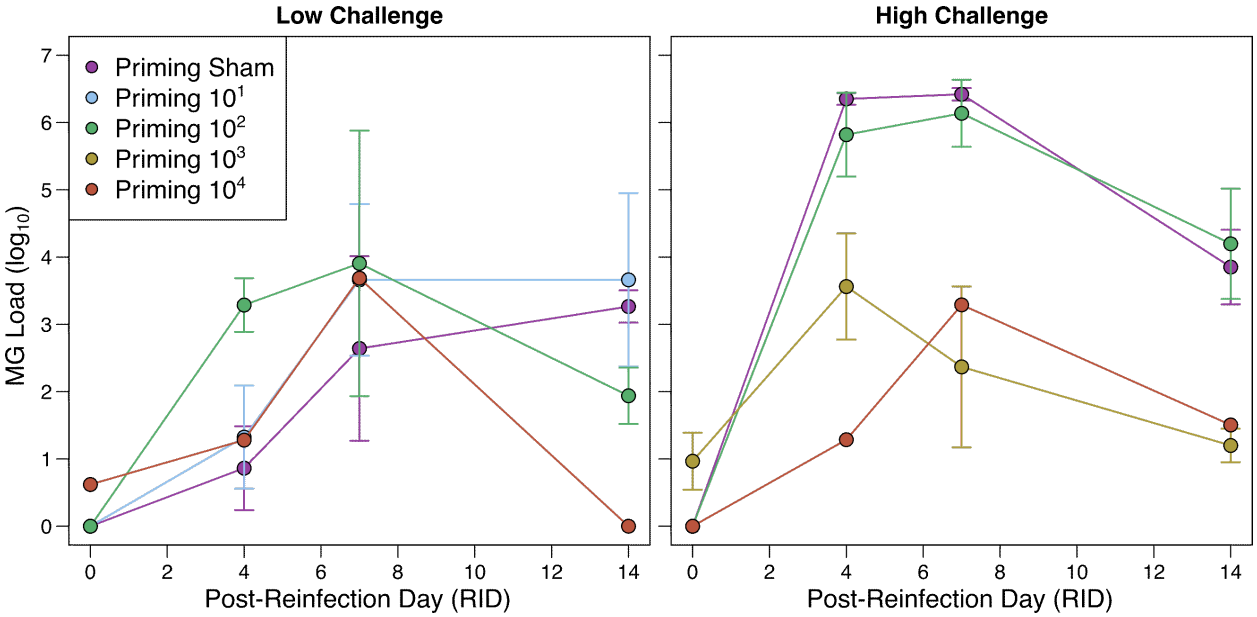


Fig S3. Pathogen load of *Mycoplasma gallisepticum* in infected birds following re-inoculation with low (left) or high (right) secondary challenge doses after a range of priming doses. Note that no birds given the lowest priming dose (10^1 CCU/mL) received a high-dose challenge. Values are averages ± standard error. Data exclude birds that did not become successfully infected from the secondary inoculation, in contrast to Fig 2 in the Main Text that includes all birds.



References

Weitzman CL, Rostama B, Thomason CA, May M, Belden LK, Hawley DM. 2021. Experimental test of microbiome protection across pathogen doses reveals importance of resident microbiome composition. FEMS Microbiol Ecol 97:fiab141.