SUPPLEMENTARY MATERIAL

Altitudinal effects on innate immune response of Turkish blind mole-rats

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Supplementary Text

Bacteria preparation

Prior to the assay, we autoclaved TSB (Tryptic Soy Broth, Sigma; 15 g broth/500 ml nanopure water) and stored it overnight at 4°C. Additionally, we reconstituted *Escherichia coli* in lyophilized 1 pellet (E-Power, *Escherichia coli* ATCC® 8739TM) into 10 ml pre-warmed 1X PBS (Sigma-Aldrich, cat. No: 02-023-1A). Using flame-sterilized forceps, we transferred the pellet to the warm PBS, incubated the solution in shaker for 30 min (37°C) to ensure hydration. Finally, we vortexed the stock solution until the pellet was completely dissolved and stored the solution for no more than 24 h at 4°C. We used this stock solution to make a working solution (10^5 colony-forming unit; CFU) by adding 1 ml of bacteria stock to 9 ml of pre-warmed PBS.

Optimization of bacteria killing assay

Before testing the bacteria killing ability of our samples, we optimized the incubation time (interval to log phase growth) and bacterial concentration. We used the concentration for E. coli 10^5 colony forming units (CFU)/ml incubated at 37°C.

Optimization of incubation time

We added 6 µl of bacteria working solution, 18 µl PBS and 125 µl TSB into two wells. We then incubated the plate in a plate shaker for 30 min at 37°C, 150 rpm and measured the absorbance at 300 nm, 340 nm, 405 nm, 490 nm, and 592 nm for 0, 4, 8, 12, 14, 20 and 24 hours to determine log-phase growth of *E. coli.*

Optimization of serum concentration

The procedure is the same as with the bacteria killing assay (below) except for this optimization we used two pooled serum samples of 4 individuals (2 from the high altitude population and 2 from low altitude populations) to ensure variation of bacteria killing ability between individuals/populations. We used serial dilutions to find the best dilution (from 1:1 to 1:128). To do this we added 18 µl (9 µl from each of two individuals) of serum to 18 µl of PBS, mixed these by pipetting, and then took 18 µl of the serum-PBS mixture to the next well. 18 µl of PBS were

added, mixed together, and then 18 µl was moved to the next row and the process was repeated until the dilution reached 1:128 (8 times). We then measured absorbance at the optimized time and wavelength (10 hrs. and 340 nm.).

Results of Optimizations

We observed log-phase growth between 5 to 12 hours of incubation at 37°C, and we selected 10 hours as our optimized incubation period for subsequent sample assays (see supplementary material, Fig. S1). Additionally, we selected the absorbance filter 340 nm because it allowed for better resolution of our data. Plasma dilution was determined to be 1:2 as this was the concentration at which 50% of bacteria were killed (Fig. S2).

Supplementary Tables

Table S1. Top set of general linear models for bacteria killing ability (BKA). Models represent the full dataset including **both sexes** (N=19, F=15, M=4). All models within Δ2 AICc of the top model are shown.

#	Models	ΔAICc Values	Weights	Df	R^2_{adj}
1	BKA_340nm ~ 1 + altitude	0.00	0.314	17	0.303
2	BKA_340nm ~ 1 + time_held	0.66	0.226	17	0.278
3	BKA_340nm ~ 1 + altitude + time_held	1.91	0.121	16	0.309

Table S2. Top set of general linear models for bacteria killing ability (BKA). Models represent the dataset including **only female** samples (N=15). All models within Δ2 AICc of the top model are shown.

#	Models	ΔAICc Values	Weights	Df	R^2_{adj}
1	BKA ~ 1 + altitude	0.00	0.579	13	0.417

Table S3. Top set of general linear models for bacteria killing ability (BKA) **with MCC variable as a covariate**. Models represent the full dataset including both sexes (N=19, F=15, M=4). All models within Δ2 AICc of the top model are shown.

#	Models	ΔAICc Values	Weights	Df	R^2_{adj}
1	BKA ~ 1 + altitude	0.00	0.512	17	0.303

Table S4. Top set of general linear models for bacteria killing ability (BKA) **with MCC variable**. Models represent the full dataset including **only female** samples (N=15). All models within Δ4 AICc of the top model are shown.

#	Models	ΔAICc Values	Weights	Df	R^2_{adj}
1	BKA ~ 1 + altitude	0.00	0.634	13	0.417

Table S5. Top set of general linear models for mean corticosterone concentrations (MCC). Models represent the full dataset including **both sexes** (N=19, F=15, M=4). All models within Δ2 AICc of the top model are shown.

#	Models	ΔAICc Values	Weights	Df	R^2_{adj}
1	MCC ~ 1	0.00	0.270	18	NA
2	MCC ~ 1 + altitude	0.69	0.191	17	0.055
3	MCC ~ 1 + sex	1.55	0.124	17	0.011
4	MCC ~ 1 + time_held	1.88	0.105	17	-0.006

Table S6. Top set of general linear models for mean corticosterone concentrations (MCC). Models represent the dataset including **only female** samples (N=15). All models within Δ2 AICc of the top model are shown.

#	Models	ΔAICc Values	Weights	Df	R^2_{adj}
1	MCC ~ 1	0.00	0.514	14	NA

Table S7. Comparisons of coccidian parasitism measures in Turkish blind mole-rats by year. Statistical results are provided for each test.

Group	Infected	Total	Prevalence	Mean intensity (SD)	Mean abundance (SD)
2017	8	8	100.0%	93.22 (95.65)	93.22 (95.65)
2018	6	11	54.5%	7.33 (6.68)	3.99 (6.08)
Statistical tests for year differences:			Two-sided p = 0.045	Bootstrap t-test with 5000 replications, p = 0.055	Bootstrap t-test with 5000 replications, p = 0.045

Table S8. Comparisons of coccidian parasitism measures in Turkish blind mole-rats by altitude. Statistical results are provided for each test.

Group	Infected	Total	Prevalence	Mean intensity (SD)	Mean abundance (SD)
Low altitude	7	11	63.6%	30.01 (44.60)	19.09 (37.22)
High altitude	7	8	87.5%	82.81 (106.40)	72.46 (102.70)
Statistical tests for year differences:			Two-sided p = 0.338	Bootstrap t-test with 5000 replications, p = 0.260	Bootstrap t-test with 5000 replications, p = 0.218

Supplementary Figures

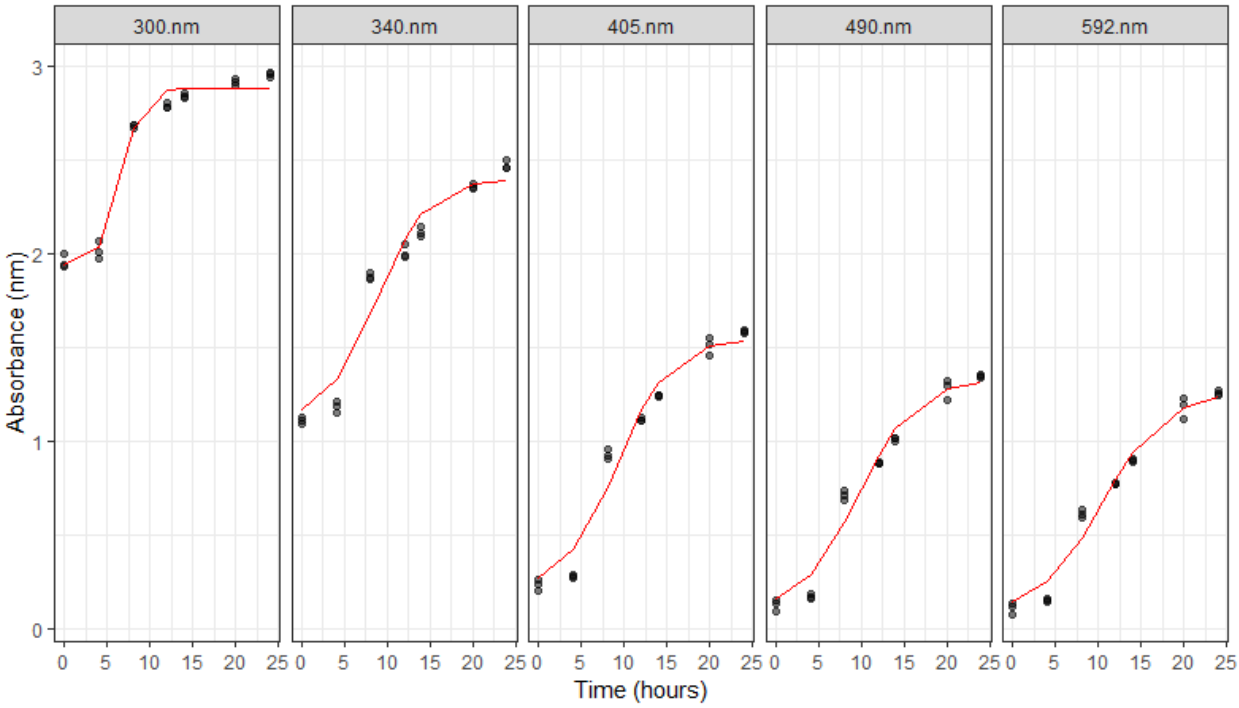

Figure S1. Bacterial growth for all absorbance filters by time (hours). The 300 nm and 340 nm absorbance filters were most effective for measuring microbial growth (i.e., a clear lag-phase, growth, and saturation curve were identifiable). The log phase (exponential) growth can be seen between 5 to 12 hours of incubation, and we selected 10 hours as our optimized incubation period. Red lines indicate predicted log-growth curves.

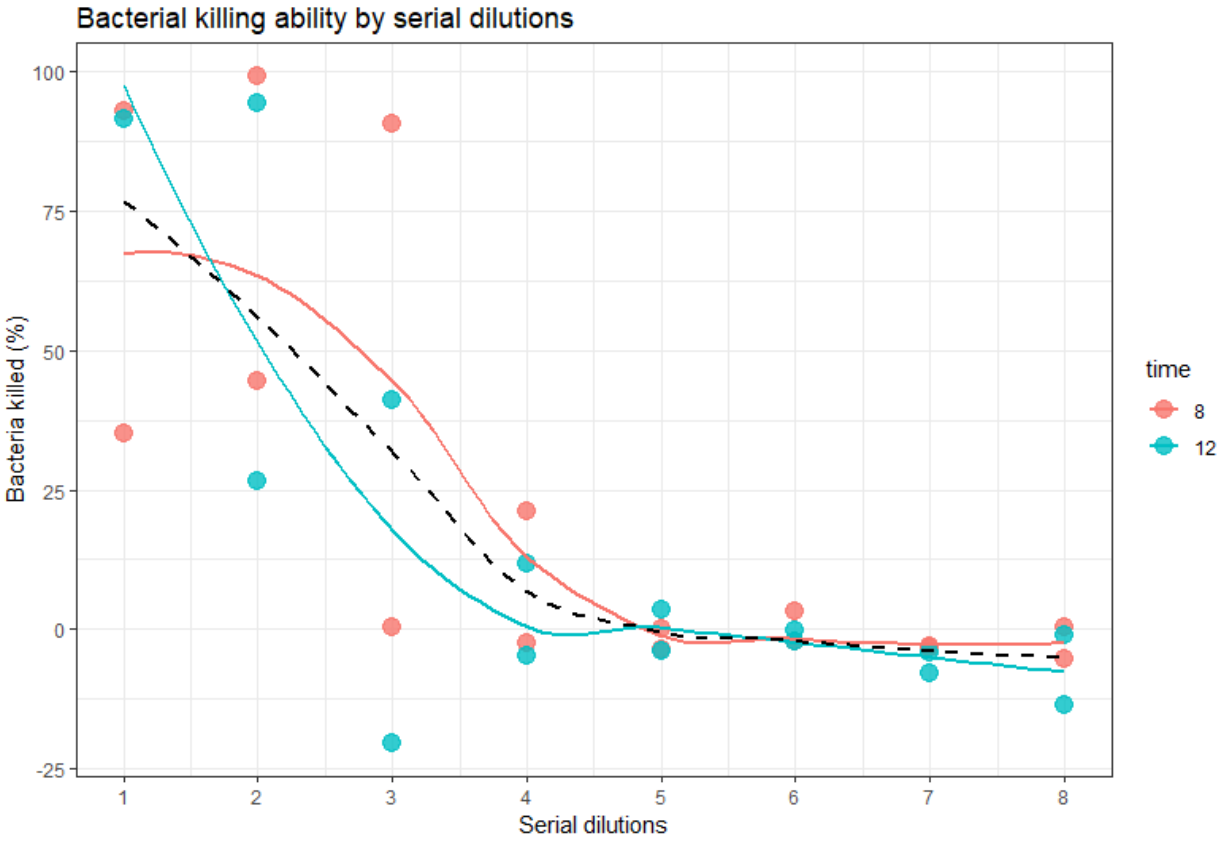

Figure S2. Bacteria killing ability by serum dilutions for 340nm. Serum dilution with 50% bacteria killing ability is optimal. Therefore, we choose the second dilution, which is closest to 50% and represents 1:2 dilution of serum. (1 = 1:1 dilution, or 2x; 2 = 1:2 dilution, or 3x, and so on). This would be a 3x dilution.