

Supplementary Information

Transovarial transmission of Yersinia pestis in its flea vector Xenopsylla cheopis

Pauling, CD, et al.

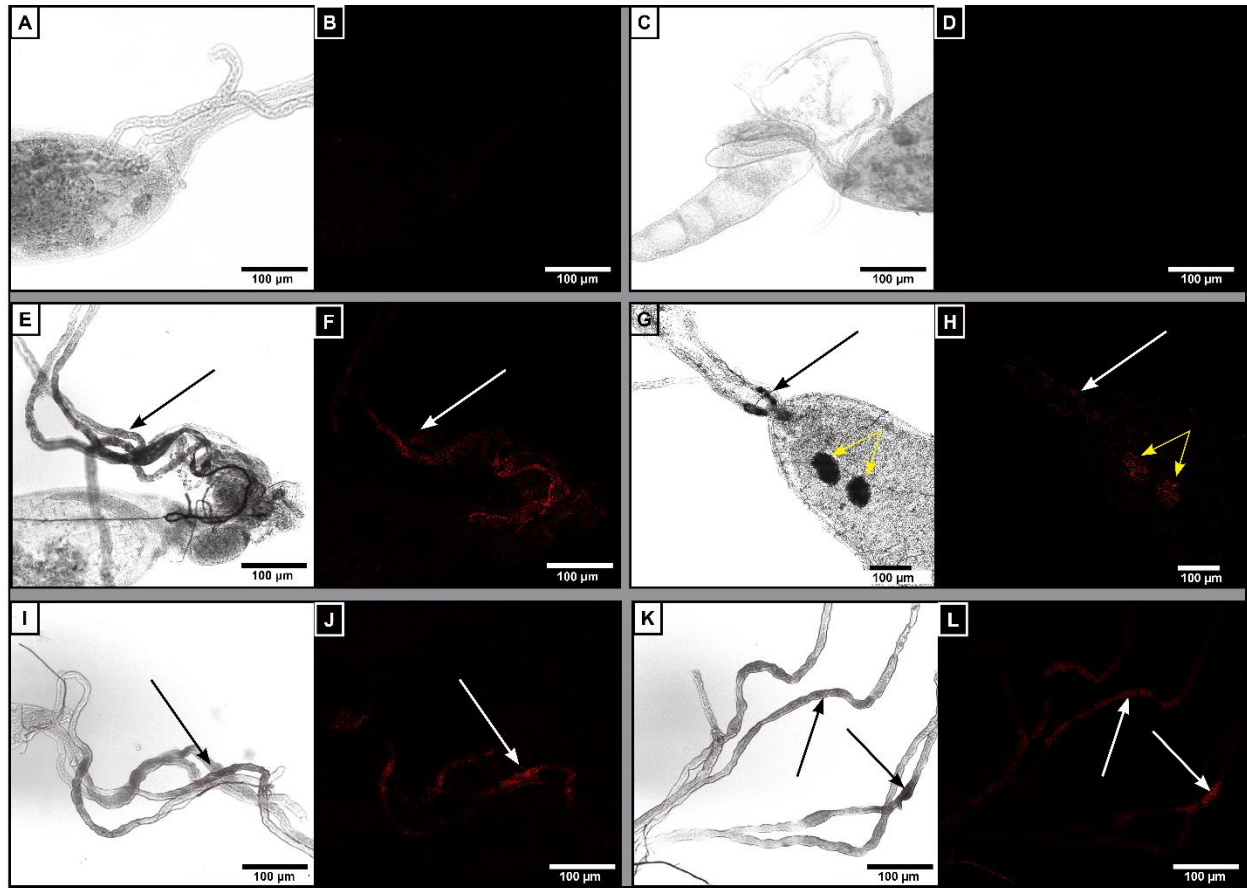
Supplemental Table S1. Summary of study design and sample numbers for each figure.

Figure: Sample	Assay	# Samples	% Positive	# Independent trials
1: Midgut/PV days 1-14				
Rat	Confocal Microscopy	59	97	3
Mice	Confocal Microscopy	58	73	3
Prairie Dog	Confocal Microscopy	54	62	3
Pig	Confocal Microscopy	59	87	3
2: Midgut/PV individual days, rat bloodmeal				
D1	Confocal Microscopy	12	100	3
D3	Confocal Microscopy	11	92	3
D7	Confocal Microscopy	10	100	3
D1	Bacterial Load Determination	27	100	3
D3	Bacterial Load Determination	27	100	3
D7	Bacterial Load Determination	15	100	3
3: Reproductive tissue, pig and prairie dog bloodmeal				
Males	Confocal Microscopy	9	56	3
Females	Confocal Microscopy	9	56	3
Females	Confocal Microscopy	10	50	3
4: Eggs				
Not infected	Confocal Microscopy	4	0	4
Infected	Confocal Microscopy	14	50	4
5: Eggs, ovarioles				
Midguts				
D1	Bacterial Load Determination	9	100	3
D3	Bacterial Load Determination	9	100	3
D7	Bacterial Load Determination	6	100	2
Eggs				
Not infected	Immunohistochemistry	20	0	5
Infected	Immunohistochemistry	25	80	5
Ovarioles				
Not infected	Immunohistochemistry	5	0	5
Infected	Immunohistochemistry	25	84	5
6: Reproductive tissue				
Not infected				
Female	TEM	15	0	2
Male	TEM	15	0	2
Infected				
Female	TEM	15	67	2
Male	TEM	15	75	2
7: Larvae				
Not infected	Confocal Microscopy	3	0	3
Infected	Confocal Microscopy	8	63	3
8: Live bacteria				
Eggs	Bacterial Load Determination	15	53	4
Larvae	Bacterial Load Determination	4	50	3
Pupae	Bacterial Load Determination	4	100	3
F1	Bacterial Load Determination	3	100	2
S1: Malpighian tubules				
Not infected	Confocal Microscopy	6	0	3
Infected	Confocal Microscopy	10	60	3
S2: Many tissues				
Infected 15 weeks	Confocal Microscopy	8	75	2
S3: Pupae				
Not infected	Confocal Microscopy	2	0	2
Infected	Confocal Microscopy	5	60	2

Supplemental Table S2. Primers used to target *Yersinia pestis* specific genes.

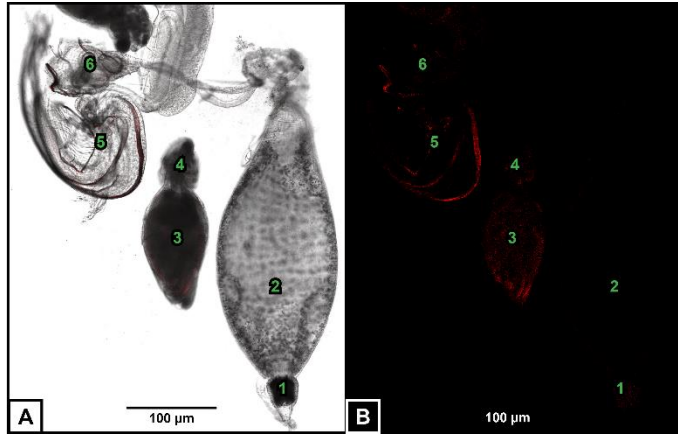
Gene	Location	Forward	Reverse	size (bp)
<i>caf1</i>	pMT1	5'-ACTCTTGTTGAACCAGCCCG-3'	5'-GGTTACGGTTACAGCATCAGTG-3'	402
<i>pla</i>	pPCP1	5'-CGGGATGCTGAGTGGAAGT-3'	5'-TATCATGTGCCCCGAACCCAG-3'	554

Supplemental Figure S1.



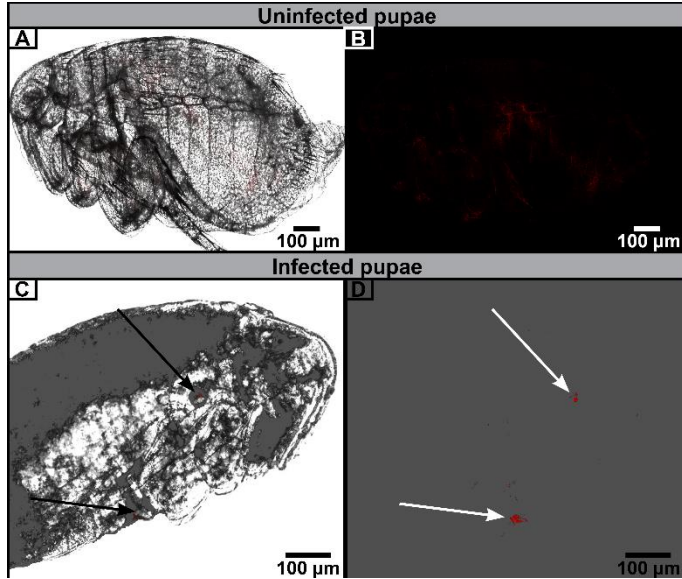
Supplemental Figure S1. *Yersinia pestis* colonizes Malpighian tubules of *X. cheopis*. Adult fleas that were not infected (A-D) or were infected with *Y. pestis* in rat (E-H) or mouse (I-L) blood, then dissected on days 5 and 10 post-infection. Representative images of Malpighian tubules: Brightfield (A, C, E, G, I, K); fluorescent channel (B, D, F, H, J, L) are shown. White arrows point to red-fluorescing bacteria in Malpighian tubules; yellow arrows point to bacteria in midgut biomasses; scale bars represent 100μm. Data were collected in two independent infection experiments, each with control and test subjects (n=6 not infected control, n=10 infected samples).

Supplemental Figure S2.



Supplemental Figure S2. Long-term bacterial presence of *Y. pestis* in reproductive tissues of *X. cheopis*. Representative images taken from tissues dissected from *Y. pestis*-infected *X. cheopis*, 15 weeks following infection in mouse blood: (A) Brightfield; (B) Fluorescent channel; anatomy is labeled as follows: 1. Proventriculus, 2. Midgut, 3. Testes, 4. Epididymis, 5. Aedeagus, and 6. Rectal papillae. Data shown were collected from two independent infection experiments (n=8 infected fleas).

Supplemental Figure S3.



Supplemental Figure S3. *Pupae from Y. pestis-infected adult X. cheopis fleas remain infected indicating transstadial transmission.* Representative confocal images of pupae from uninfected (A-B) or *Y. pestis*-infected (C-D) adult *X. cheopis* fleas that were removed from cocoons: (A, C) Brightfield merged with fluorescent channel; (B, D) Fluorescent channel. Arrows connect the bacteria to the corresponding region in the pupa. Data were collected from two independent infection experiments, each with control and test subjects (n=2 not infected control pupae, n=5 infected pupae).