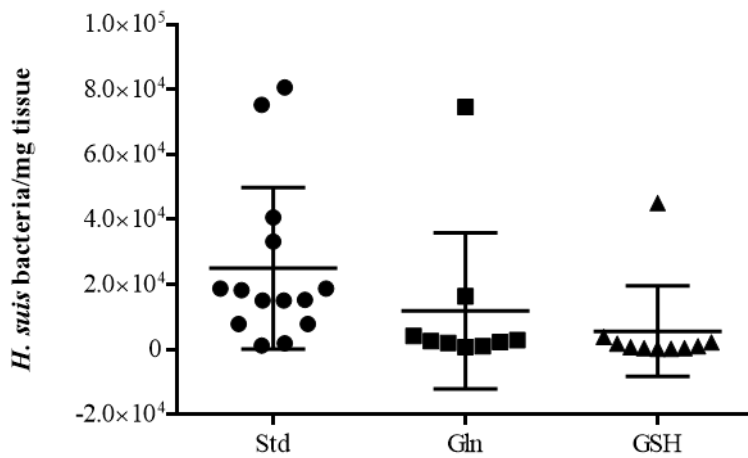


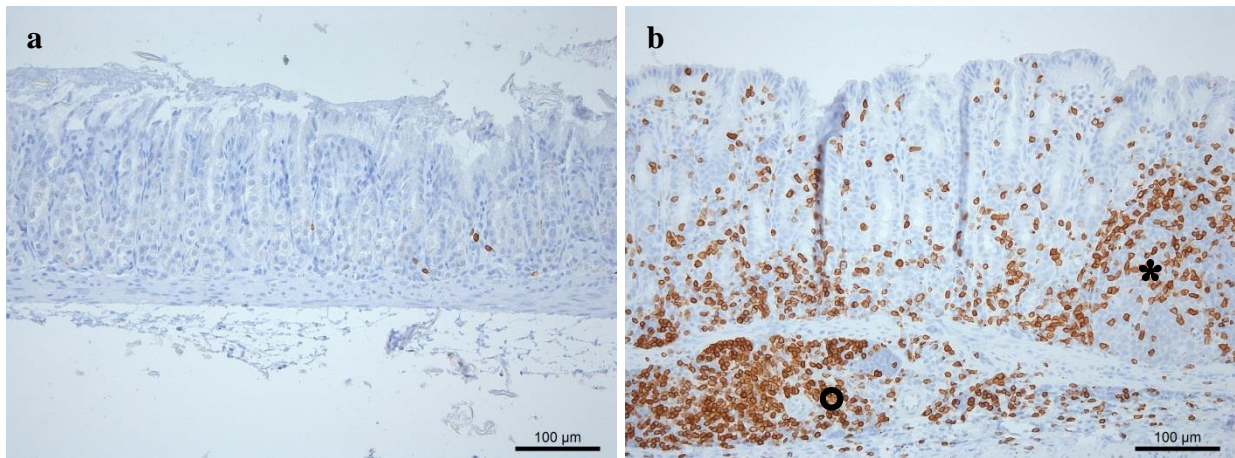
1 **ORAL GLUTATHIONE SUPPLEMENTATION DRASTICALLY REDUCES**
2 ***HELICOBACTER*-INDUCED GASTRIC PATHOLOGIES**

3 Ellen De Bruyne, Richard Ducatelle, Dennis Foss, Margaret Sanchez, Myrthe Joosten, Guangzhi
4 Zhang, Annemieke Smet, Frank Pasmans, Freddy Haesebrouck, Bram Flahou

5
6 **Figure S1:** *Helicobacter suis* colonization in the antrum of experimentally infected Mongolian
7 gerbils receiving a standard diet (Std), glutamine-supplemented diet (Gln) or glutathione
8 supplemented diet (GSH).

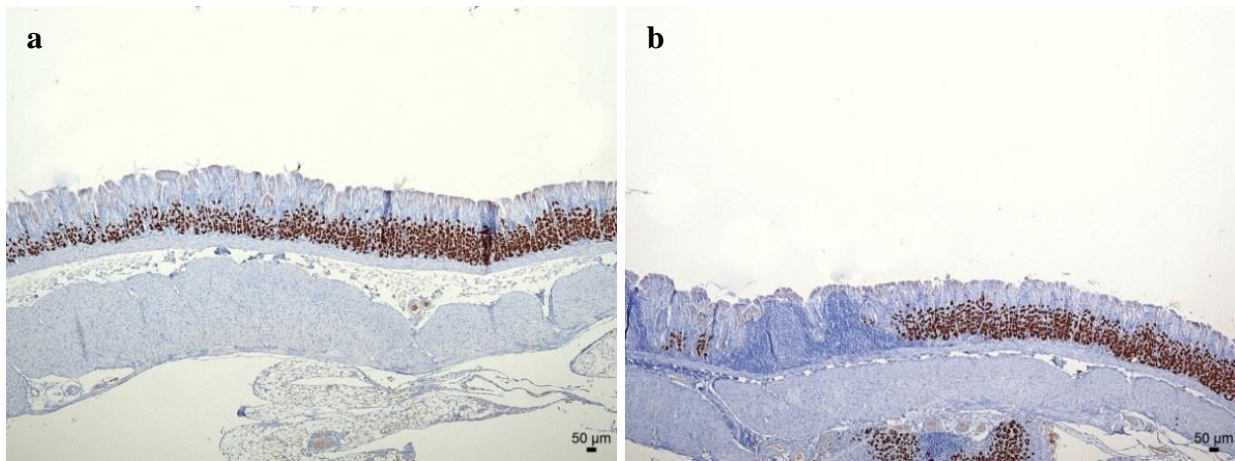


11 **FIGURE S2:** CD3 staining of the antrum of a control animal (A) and a *Helicobacter suis* infected
12 animal (B), both receiving the standard diet. CD3 positive T-cells (brown) are only present in low
13 numbers in the antrum of control animals receiving the standard diet (A). In contrast, they are
14 abundantly present in the antrum of *Helicobacter suis* infected animals receiving the standard diet
15 (B). Superficial lymphoid aggregates (*) contain more CD3 negative T-cells, whereas deep
16 lymphoid aggregates (°) are mainly composed of CD3 positive cells.

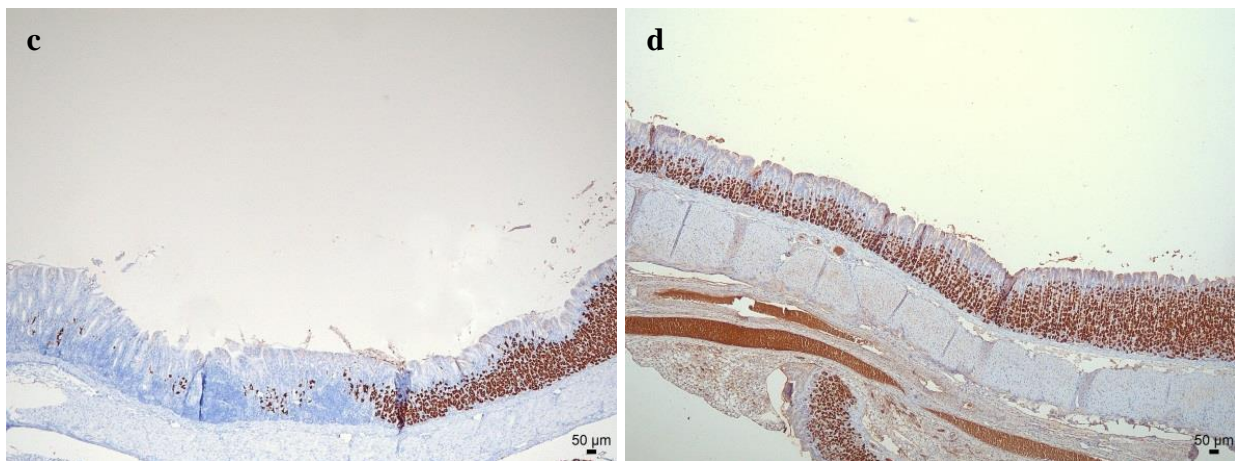


19 **FIGURE S3:** Immunohistochemical staining of the parietal cell hydrogen potassium ATPase. H^+ ,
20 K^+ ATPase-positive parietal cells (brown) in the antrum/antrum-corporum transition zone. A: control
21 animals receiving the standard diet; B: *H. suis* infected animal with the standard diet; C: *H. suis*
22 infected animal receiving the glutamine-supplemented diet; D: *H. suis* infected animal receiving
23 the glutathione-supplemented diet. An abrupt loss of parietal cells is observed in *H. suis* infected
24 animals receiving the standard diet and *H. suis* infected animals receiving the Gln-supplemented
25 diet, but not in *H. suis* infected animals receiving the GSH-supplemented diet.

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29 **Table S1:** The fold change of the metabolites that were significantly different in Minimal
 30 Essential Medium (MEM) and/or Mueller Hinton Medium (MH) after 12, 18 and 24 hours of
 31 incubation with *Helicobacter suis* compared to the 2 hour incubation point. Highlighted in green
 32 are the metabolites that significantly decreased over time, highlighted in red are the values that
 33 significantly increased. The p-value is given of each measurement.

Name	MEM						MH					
	Hour 12 / Hour 2		Hour 18 / Hour 2		Hour 24 / Hour 2		Hour 12 / Hour 2		Hour 18 / Hour 2		Hour 24 / Hour 2	
	Fold Chan ge	P- value	Fold Chan ge	P- value	Fold Chan ge	P- value	Fold Chan ge	P- value	Fold Chan ge	P- value	Fold Chan ge	P- value
Serine	0.50	0.00	0.24	0.00	0.07	0.00	1.01	0.74	0.99	0.84	0.95	0.27
Betaine	1.11	0.25	1.20	0.04	1.28	0.02	0.99	0.48	0.98	0.47	1.01	0.75
Alanine	0.71	0.00	0.49	0.00	0.27	0.00	1.03	0.09	1.05	0.09	1.00	0.97
Beta-alanine	0.85	0.46	0.99	0.80	1.18	0.39	1.05	0.64	0.97	0.95	1.11	0.60
Aspartate	0.36	0.00	0.19	0.00	0.12	0.00	0.45	0.00	0.23	0.00	0.13	0.00
Asparagine	0.02	0.00	0.02	0.00	0.02	0.00	0.20	0.00	0.05	0.00	0.03	0.00
Glutamate	2.59	0.00	3.60	0.00	3.35	0.00	1.04	0.27	1.06	0.18	1.05	0.30
Glutamine	0.41	0.00	0.11	0.00	0.00	0.00	0.64	0.00	0.34	0.00	0.12	0.00
3-methyl-2-oxovalerate	3.94	0.00	5.23	0.00	5.75	0.00	1.92	0.00	2.34	0.00	2.51	0.00
Isoleucine	0.92	0.06	0.90	0.03	0.92	0.01	1.05	0.19	1.04	0.42	1.01	0.80
Leucine	0.91	0.02	0.89	0.02	0.90	0.01	1.06	0.10	1.06	0.17	1.02	0.62
Valine	0.97	0.46	0.89	0.08	0.89	0.04	1.06	0.14	1.06	0.09	1.04	0.32
Cysteine	0.21	0.00	0.18	0.00	0.16	0.00	0.57	0.01	0.36	0.00	0.26	0.00
N-acetylcysteine	0.82	0.10	0.71	0.02	0.97	0.81	0.71	0.18	0.44	0.00	0.47	0.00
Cystine	1.76	0.11	2.88	0.00	2.87	0.00	2.04	0.01	2.61	0.00	2.82	0.00
Methionine	0.88	0.02	0.83	0.01	0.81	0.00	1.08	0.08	1.07	0.12	1.01	0.77
Ornithine	0.87	0.11	0.81	0.01	0.91	0.21	1.00	0.96	0.98	0.84	1.06	0.46
Proline	0.34	0.00	0.01	0.00	0.01	0.00	0.99	0.86	1.02	0.74	0.97	0.28
N-acetylornithine	0.90	0.57	1.11	0.48	0.98	0.79	1.00		1.00		1.00	
Creatine	1.03	0.52	1.24	0.00	1.29	0.04	0.92	0.26	0.94	0.35	0.94	0.38
Glutamylglutamate	1.00		1.00		1.00		0.96	0.53	1.00	0.98	0.80	0.01
Prolylleucine	1.00		1.00		1.00		1.01	0.58	1.00	0.91	0.95	0.04

Leucylproline	1.00		1.00		1.00		0.53	0.10	1.07	0.72	0.41	0.02
Pro-hydroxy-pro	0.91	0.26	0.89	0.12	0.72	0.02	1.00		1.00		1.00	
Threonylphenylalanine	1.00		1.00		1.00		1.13	0.01	1.07	0.04	1.04	0.54
Carnosine	1.10	0.34	1.10	0.21	1.14	0.06	1.26	0.03	1.20	0.19	1.34	0.03
Fructose	0.99	0.83	1.05	0.44	1.09	0.02	1.03	0.32	1.09	0.01	1.01	1.00
Maltose	1.00		1.00		1.00		1.78	0.00	1.83	0.00	1.89	0.02
Sorbitol	0.70	0.04	0.99	0.96	1.03	0.73	1.45	0.13	1.16	0.41	0.98	0.79
Glycerate	1.13	0.00	1.21	0.04	1.35	0.03	1.15	0.12	1.27	0.02	1.20	0.06
Glucose	0.94	0.38	1.04	0.60	1.00	0.96	1.58	0.00	1.75	0.00	1.89	0.04
Pyruvate	0.58	0.12	0.34	0.05	0.14	0.00	0.59	0.05	0.27	0.00	0.26	0.00
Lactate	0.04	0.00	0.03	0.00	0.05	0.00	0.03	0.00	0.03	0.00	0.04	0.00
Arabitol	0.78	0.13	0.87	0.33	0.97	0.84	1.27	0.05	1.07	0.43	1.01	0.92
Ribitol	0.37	0.00	0.41	0.01	0.35	0.00	0.84	0.38	0.67	0.06	0.55	0.05
Ribose	0.69	0.02	0.86	0.37	1.03	0.87	1.00		1.00		1.00	
Xylonate	2.62	0.00	3.37	0.00	3.94	0.00	1.23	0.09	1.18	0.17	1.18	0.17
Citrate	1.20	0.22	1.44	0.10	1.50	0.03	1.13	0.03	1.11	0.01	1.01	0.89
Succinate	0.12	0.00	0.10	0.00	0.08	0.00	3.53	0.00	3.50	0.00	2.49	0.00
Fumarate	1.00		1.00		1.00		1.03	0.62	0.81	0.18	0.61	0.00
Malate	1.00		1.00		1.00		0.89	0.09	0.53	0.00	0.27	0.00
Oxaloacetate	1.01	0.83	1.08	0.47	0.80	0.76	1.81	0.20	1.01	0.99	1.53	0.27
Phosphate	0.37	0.00	0.23	0.00	0.16	0.00	0.97	0.28	0.93	0.04	0.92	0.02
Dihomo-linolenate	1.93	0.00	2.49	0.00	2.92	0.00	1.37	0.03	1.58	0.07	1.84	0.00
Docosapentaenoate	2.05	0.04	2.16	0.04	2.87	0.01	1.26	0.05	1.47	0.11	1.64	0.02
Docosaheptaenoate	1.69	0.02	1.83	0.01	2.20	0.00	1.29	0.11	1.39	0.09	1.49	0.04
Isovalerate	1.00		1.00		1.00		1.57	0.04	7.39	0.00	16.9	0.00
Caproate	1.20	0.39	1.42	0.24	1.38	0.38	1.08	0.96	1.51	0.10	2.34	0.02
Caprylate	1.35	0.07	1.56	0.06	2.05	0.00	2.11	0.00	2.20	0.00	2.78	0.00
5-dodecenoate	1.00		1.21	0.39	1.63	0.00	1.30	0.08	1.57	0.00	1.99	0.00
Myristoleate	3.15	0.01	4.61	0.01	7.36	0.00	3.10	0.00	4.90	0.00	6.06	0.00
Arachidonate	2.13	0.01	2.31	0.00	2.91	0.00	1.37	0.01	1.59	0.03	1.76	0.00
Ethanolamine	1.68	0.05	1.72	0.01	2.30	0.00	0.91	0.53	1.05	0.83	1.25	0.14
Glycerophosphorylcholine	1.44	0.10	1.80	0.03	2.00	0.02	0.92	0.03	0.94	0.07	0.98	0.45
Myo-inositol	1.02	0.73	1.03	0.21	1.08	0.04	1.18	0.07	1.08	0.43	1.07	0.92

1-palmitoyl-glycerophosphocholine	0.93	0.99	1.46	0.16	1.08	0.86	1.69	0.02	1.59	0.03	1.04	0.12
1-stearoyl-glycerophosphocholine	0.93	0.88	1.45	0.17	1.19	0.71	1.75	0.01	1.75	0.01	1.42	0.11
1-oleoyl-glycerophosphocholine	0.84	0.81	1.41	0.20	1.12	0.82	1.78	0.02	1.60	0.04	1.43	0.18
1-arachidonoylglycerophosphocholine	0.71	0.65	1.81	0.12	1.18	0.64	1.61	0.04	1.33	0.17	1.15	0.51
Xanthine	1.17	0.00	1.23	0.00	1.34	0.00	0.93	0.50	0.89	0.30	0.90	0.32
Xanthosine	1.02	0.58	1.16	0.01	1.25	0.01	0.92	0.10	0.95	0.39	0.83	0.02
Hypoxanthine	1.69	0.00	2.06	0.00	2.42	0.00	1.00		1.00		1.00	
Inosine	1.16	0.01	1.10	0.04	1.08	0.10	0.78	0.09	0.77	0.16	0.58	0.00
2'-deoxyinosine	0.94	0.12	0.89	0.02	0.81	0.00	1.00		1.00		1.00	
Adenine	0.93	0.30	0.94	0.13	0.86	0.09	1.02	0.80	0.85	0.14	0.84	0.09
Adenosine	0.03	0.00	0.05	0.00	0.04	0.00	1.00		1.00		1.00	
Adenosine 3'-monophosphate	1.00		1.00		1.00		1.44	0.02	1.51	0.01	1.36	0.03
Adenosine 5'-monophosphate	2.19	0.00	2.48	0.00	1.74	0.01	1.17	0.03	1.34	0.00	1.39	0.00
Guanine	1.98	0.00	2.15	0.00	2.21	0.00	0.65	0.07	0.51	0.03	0.59	0.04
Guanosine	0.91	0.26	0.96	0.47	0.89	0.23	1.44	0.01	1.19	0.21	1.10	0.44
2'-Deoxyguanosine	0.73	0.03	0.70	0.01	0.55	0.01	1.00		1.00		1.00	
1-methylguanosine	0.93	0.49	0.97	0.83	0.94	0.61	1.00		1.00		1.00	
Urate	0.76	0.16	0.71	0.03	0.63	0.02	0.98	0.90	0.94	0.50	0.84	0.17
Uridine	0.99	0.86	1.03	0.47	0.98	0.75	1.20	0.18	1.25	0.14	1.30	0.02
Nicotinamide	1.06	0.23	1.25	0.02	1.04	0.76	1.13	0.32	1.56	0.02	1.34	0.07
Nicotinamide adenine dinucleotide	0.25	0.00	0.25	0.00	0.25	0.00	0.26	0.00	0.23	0.00	0.23	0.00
Adenosine 5'diphosphoribose	0.77	0.06	0.77	0.06	0.77	0.06	1.03	0.86	0.74	0.05	0.63	0.01
Trigonelline	0.37	0.00	0.27	0.00	0.27	0.00	1.48	0.33	1.04	0.99	1.24	0.43

Pantothenate	0.97	0.77	1.04	0.54	0.91	0.40	1.18	0.02	0.99	0.81	1.05	0.36
Hippurate	1.04	0.29	1.05	0.21	1.07	0.04	1.04	0.48	1.05	0.36	1.00	0.93
Glycolate	0.60	0.09	1.17	0.41	0.72	0.26	1.56	0.04	1.26	0.28	1.22	0.25
Phenol sulfate	1.02	0.67	0.99	0.86	1.10	0.01	0.95	0.68	1.06	0.60	1.06	0.48
Daidzein	1.00		1.00		1.00		1.18	0.02	1.31	0.00	1.28	0.00
Genistein	1.00		1.00		1.00		1.12	0.00	1.04	0.11	1.08	0.07
Erythritol	0.97	0.68	0.94	0.46	1.01	0.87	1.11	0.00	1.13	0.07	1.04	0.64

35 **Table S2:** An overview of the average feed intake per gerbil per day and the average weight gain
 36 per gerbil per week in the non-infected control groups (Control) and in gerbils experimentally
 37 infected with *Helicobacter suis* (Infected), fed a non-supplemented diet (Std), a diet
 38 supplemented with 5% glutamine (Gln) or a diet supplemented with 0.8% glutathione (GSH).
 39 Weight is expressed in grams \pm standard deviation.

	Diet	Estimated average feed intake per gerbil per day	Average weight gain per gerbil per week
Control	Std	3.4	1.50 \pm 0.16
	Gln	3.9	1.65 \pm 0.12
	GSH	3.8	1.08 \pm 0.21
Infected	Std	3.6	1.43 \pm 0.31
	Gln	3.7	1.53 \pm 0.43
	GSH	3.9	1.33 \pm 0.30

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42 **TABLE S3:** Overall inflammation in the stomach of gerbils experimentally infected with
 43 *Helicobacter suis*. ¹The gerbils received either a standard diet, a standard diet supplemented with
 44 5% glutamine or a standard diet supplemented with 0.8% glutathione. ²Inflammation scores: 0 =
 45 no inflammation; 1 = mild diffuse infiltration of lymphocytes or presence of one small aggregate
 46 of inflammatory cells; 2 = moderate diffuse infiltration and/or the presence of 2-4 inflammatory
 47 aggregates; 3 = marked diffuse infiltration and/or the presence of at least 5 inflammatory
 48 aggregates. ³Displayed are the number of animals out of ten animals in each infected group with a
 49 specific overall inflammation score.

Groups¹	Inflammation score antrum				Inflammation score corpus			
	0²	1	2	3	0	1	2	3
Standard	0 ³	0	4	6	7	3	0	0
Glutamine	1	2	4	3	8	2	0	0
Glutathione	7	3	0	0	9	1	0	0