Supplemental Material

Helicobacter pylori colonization critically depends on postprandial gastric conditions

Roland Bücker[1], Marina Azevedo-Vethacke[1], Claudia Groll[1], Désirée Garten[1], Christine Josenhans[2], Sebastian Suerbaum[2*] and Sören Schreiber[1*]

[1]*Institut für Physiologie, Ruhr-Universität Bochum, Im Lottental 36, 44801 Bochum, Germany*

[2]*Institut für Medizinische Mikrobiologie und Krankenhaushygiene, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, 30625 Hannover, Germany*

Short Title: The first minutes of *H. pylori* infection

*Two corresponding authors listed for the paper. Email: soeren.schreiber@rub.de (S. Schreiber); suerbaum.sebastian@mh-hannover.de (S. Suerbaum)

Corresponding author for submission process:

Soeren Schreiber
Institut fuer Physiologie
Ruhr-Universitaet Bochum
Im Lottental 36
44801 Bochum, Germany
phone.:+49 -234 -32 29126
fax.: +49 -234 -32 14191
e-mail: soeren.schreiber@rub.de

Supplemental Figure Legends

Figure S1

Schematic representation of the gerbil (A) and the gerbil stomach (B) in the setting. (A) The luminal perfusion that insures microaerobic conditions is shown over the incised stomach. On both sides of the laparatomy cut the supply and the removal of the peritoneal dialysis are displayed. (B) The different anatomic regions of the gerbil's stomach and the incision of the ventral wall of the antrum region are shown together with the side of nanosample collection and inoculation of *H. pylori*.

Figure S2

Luminal and juxtamucosal densities of *H. pylori* in the three age-dependent gastric profiles. (A) Original uncoloured image of Figure 3. Left panels: Luminal samples analyzed in a heated chamber. The visible volume is 200 picoliters. Right panels: Nanosamples from the anesthetized gerbil, with *H. pylori* colonizing the juxtamucosal mucus. The profile simulating the postprandial gastric conditions in young children causes a remarkably higher colonization. Five nanosamples of juxtamucosal mucus were averaged to determine the colonization density of *H. pylori* in each animal. **(B)** Original uncoloured image of Figure 1 B.

Figure S3

Correlation of gastric pH, age, food-intake and *H. pylori* acquisition. The susceptibility to *Helicobacter pylori* infection could depend on age- and food-dependent postprandial reacidification patterns. Young children (after ingestion of any kind of food) and schoolchildren (after ingestion of a milk-based meal that features a high buffer value) might have the highest probability of *H. pylori* infection. The corresponding age groups are highlighted in red. The danger of *H. pylori* infection in babies and adults as well as in schoolchildren after ingestion of a meal with a mean buffer value is proposed to be lower. The corresponding age groups are marked in green.

Supporting Information

Acid secretion is age-dependent

In the human stomach, the amount of secreted acid increases according to age. The basal gastric lumen pH ranges from 4 in preterm infants and neonates [1-3] to a mean pH of 1.5 in adults [4]. In the neonate, the acid secretion rate is 0.03 mequi · h^{-1} and raises up to 0.5 mequi · h^{-1} at half a year [5]. At an age of 1- 2 years, the maximal acid secretion rate is approx. 2 mequi · h^{-1} [6, 7] and 5 - 15 mequi · h^{-1} in young schoolchildren [5, 7, 8]. This continuous increase comes to a halt at the end of adolescence, when the mean maximal acid output has finally reached the adult value of 30 mequi· h^{-1} [9] (0.2 - 0.4 mequi · h^{-1}· kg^{-1} [5, 10]). Comparable to the increase of acid secretion, the secretion of pepsin also rises [5]. Pepsin, in particular pepsin C, is a decisive component of the gastric antibacterial barrier and causes a rapid loss of bacterial motility which influences the possibility of bacterial colonization [11, 12].

The pH in the gastric lumen

After a meal, the luminal pH is different within the regions of the stomach. The fundus/corpus region of the stomach with lower motility stores and acidifies the chymus, however, the antral pump mixes the chymus through propulsion and retropulsion movements. After a meal the postprandial pH elevation is most prominent in the fundus/corpus region whereas in the antrum region the postprandial pH-changes are flattened [13-15]. The buffering of the gastric content after a meal is not uniform, layers of different acidity are detectable [16] and at the gastroesophageal junction [17] a poorly buffered acidic region occurs in the postprandial phase.

Postprandial pH profiles

The course of the postprandial pH reduction depends on the pH and buffer value of the food. For a comparable measurement of the postprandial pH course, test meals [18] can be used. However, to estimate representative postprandial pH values from the normal course of life, we used summarized data from gastric 24 hour pH-metries.

Babies -postprandial profile B-

In the first series of measurements, the postprandial pH profile of a milk-fed baby was simulated (profile **B**). This profile is characterized by a time period of half an hour in which the luminal pH remained at the initial neutral pH, followed by a slow pH decrease [19, 20]. Due to the neutral gastric lumen pH pepsins are not active. The pH value in the gastric lumen was kept at 7.0 for 30 minutes, which under these conditions is the maximal time period, in which ingested *H. pylori* remained motile.

The mean pH of homogenized and pasteurized cow's milk is 6.7 [21], while the pH of human breast milk is 7.4 [22]. Since pepsins are inactive at these pH values, the hydrolysis of milk is slow [23, 24]. After 48 min for human milk and 78 min for infant formula, half of the liquid gastric content had left the stomach (gastric half emptying time) [25], partly uncoagulated milk leaves the stomach. The buffer value of human milk (7 mmol $\cdot$ l^{-1} $\cdot$ pH^{-1} [26, 27]) is lower compared to cow's milk (25 mmol $\cdot$ l^{-1} $\cdot$ pH^{-1} [26, 27]). Thus, a breast-fed infant shows a stable neutral pH in the gastric lumen for the first half hour, followed by a slow decrease to nearly pH 5 in 90 minutes after feeding [20, 28]. At the age between 6 and 12 months following weaning, the reacidification only slowly becomes steeper, due to the higher buffer value of cow's milk or dairy products which are usually consumed at this age. The postprandial pH profile in this age group is characterized by a mean gastric pH of 6 half an hour after the meal and pH values between 3.5 and 5.5 after one hour [29, 30]. The same low colonization rate observed with profile **B** at pH 7.0 (breast feeding) was also measured at a pH of 6.5 (bottle feeding with infant formula) (n = 2 animals, colonisation rates 1710:1 and 1800:1). In addition, the postprandial pH in premature infants also fits to the profile **B** [31].

Young children -postprandial profile C-

The second series simulated the postprandial pH profile of infants and young children in the age range between 1 year and young schoolchildren after milk or milk-based meals (profile **C)**. The profile was reproduced during the period of 15 min from pH 7 to pH 4, in which the ingested *H. pylori* remain motile in the gastric juice. Starting at a neutral pH of 7.0, the gastric

lumen pH decreased to pH 6.0 within 2 min, in 5 min it was at pH 5.0, and lowered to pH 4.0 a further 7 min later [21, 32].

In infants and young children the peak acid output (comparable to postprandial acid output [33]) is correlated to the body weight [10] and body surface [6]. Due to the mean pH of 6.7 and the high buffer value of cow's milk, the gastric content of the infant and even the young schoolchild after a milk or milk-based meal is buffered to the pH between 6 and 7. The higher values of activated pepsin immediately clot the milk [34] and the pH decreases. Within several minutes the range of pH 4 is reached and the profile is flattened. Therefore, one hour after a milk meal the pH is still in the region of pH 4 [35]. One hour after a mixed diet of other food components, the profile of young infants still ends at pH 4, however, in schoolchildren that ate a similar meal, the luminal pH after one hour reached lower values [35].

Even in adults, the high buffer value of 250 ml milk (> 20 mmol $\cdot$ l^{-1} $\cdot$ pH^{-1} [21, 26]) increases the luminal pH to 5.5 and the time period to lower the lumen pH below 3 is 20 min [36].

Adults -postprandial profile A-

In the third series of experiments the postprandial pH profile of an adult was simulated, starting with a gastric luminal pH of 5.0 and lowering the pH over a time period of 15 min, which is the time point at which >90% of the ingested bacteria have lost motility. The profile started at pH 5.0, reaching the values of pH 4.0, 3.0 and 2.0 at 5, 10, and 15 minutes [15, 37-40] (profile **A**).

In schoolchildren between 6 and 14 years, the maximal acid secretion rate (MAO) is lower [10] and the basal amount of free acid in the stomach is half of the adult values [41], nevertheless, in particular older schoolchildren show postprandial pH profiles comparable to adults [42].

Meals with increased buffer values und a high fraction of the solid phase flatten the A profile in the region below pH 3 [43]. Highly buffering antacids, however, flatten the postprandial pH profile of adults into a profile similar to the infant profile **C** [36, 44, 45].

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Figure S1

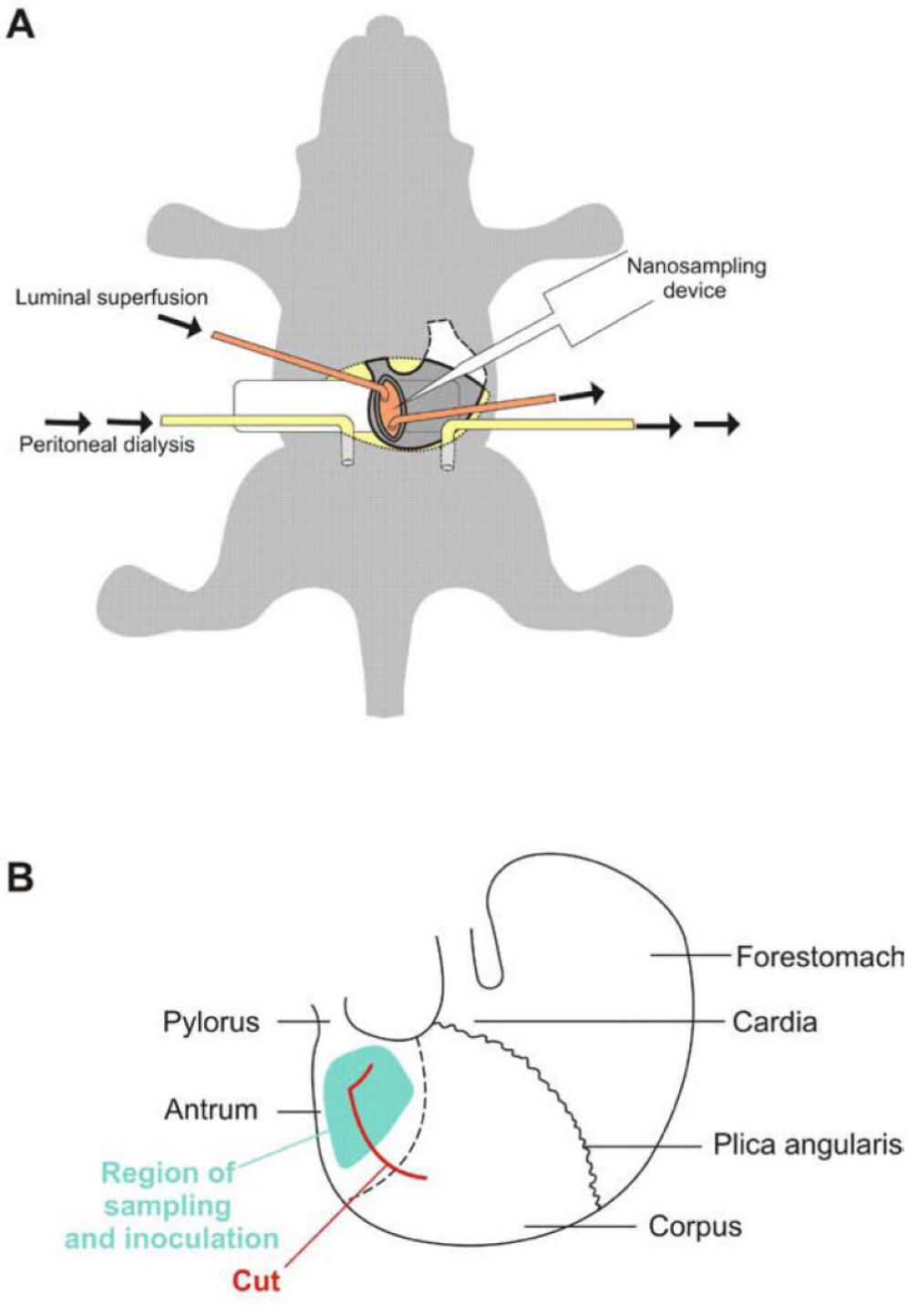
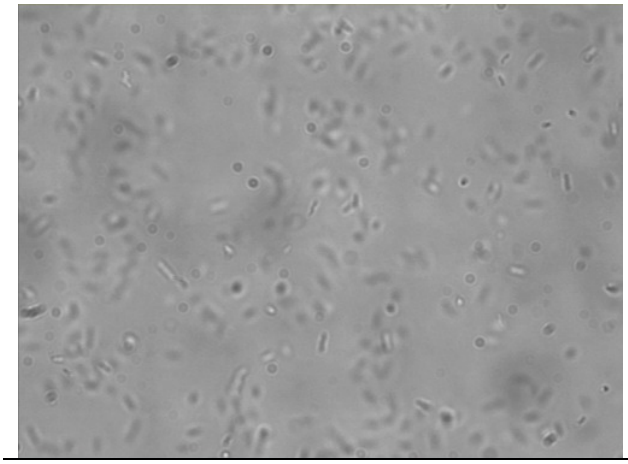
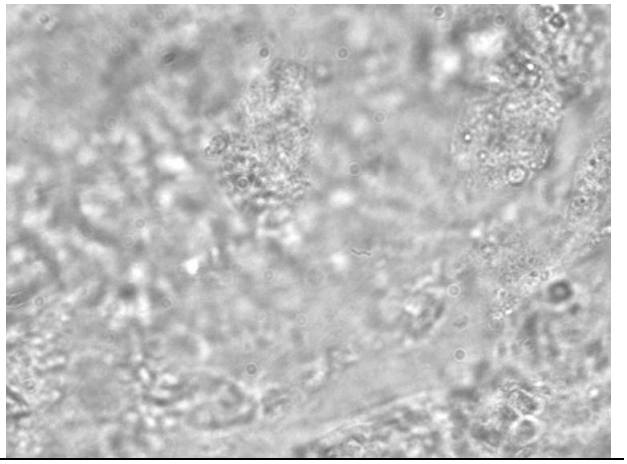

A
Luminal superfusion
Nanosampling device
Peritoneal dialysis
B
Forestomach
Pylorus
Cardia
Antrum
Plica angularis
Region of sampling and inoculation
Corpus
Cut

Figure S2 (A) Uncoloured original image of Figure 3

Luminal *Helicobacter pylori* numbers and age-dependent postprandial mucus colonization of *H. pylori*

Lumen sample (visible 200 pl)	Juxtamucosal mucus layer (approx. 1,000 μm^2)
Profile B (postprandial "Baby")	
	
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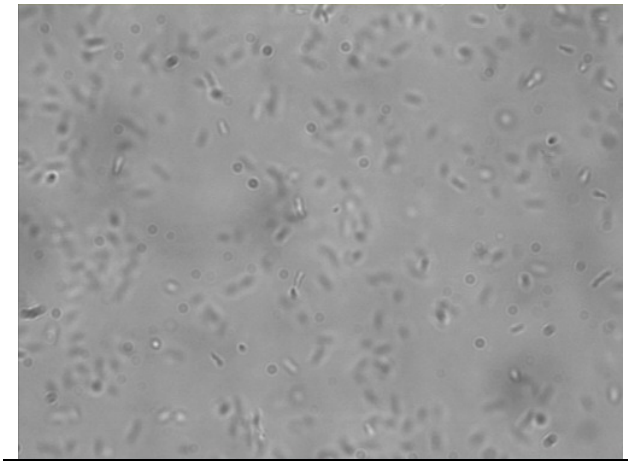	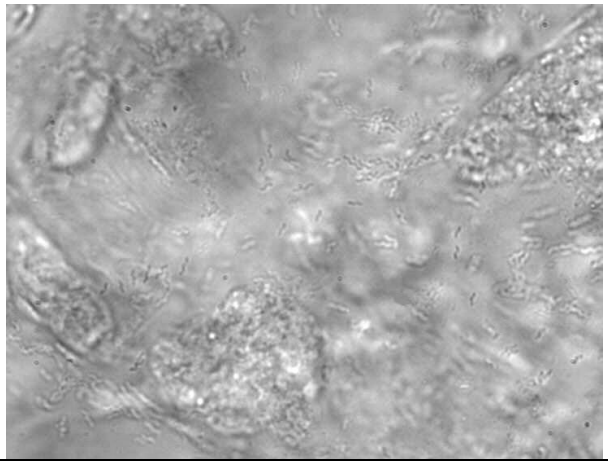
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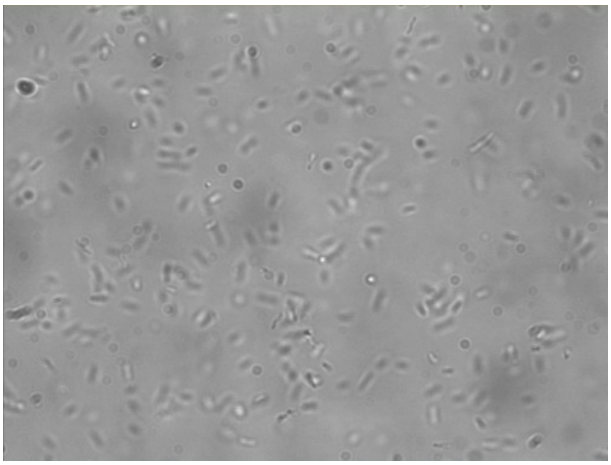
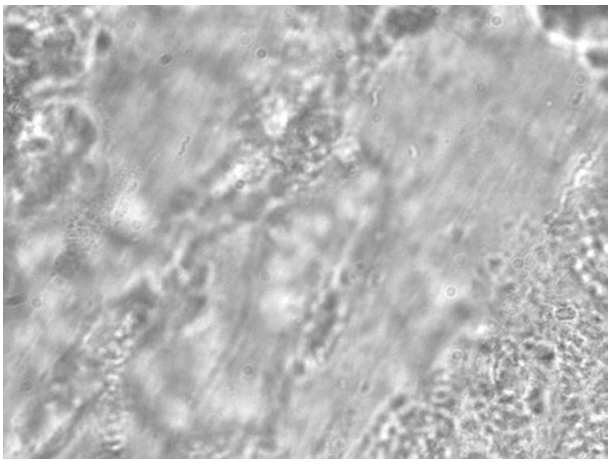
	

Figure S2 (B) Uncoloured original image of Figure 1 (B)

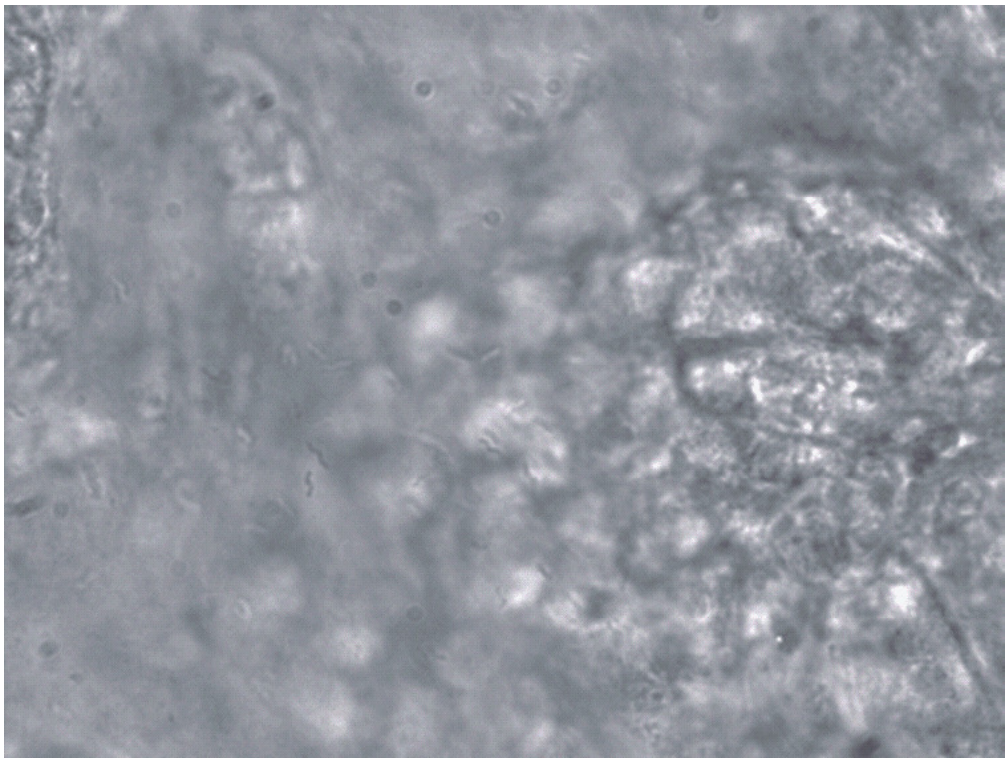
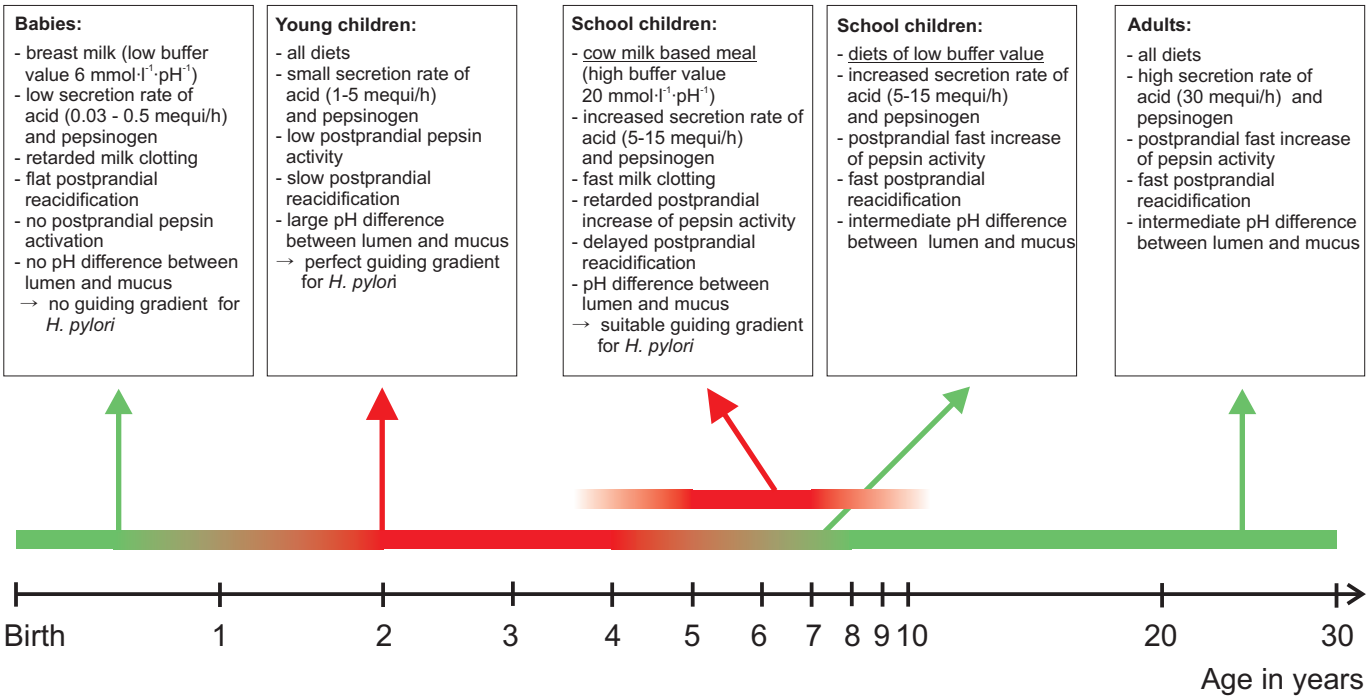


Figure S3


Babies:
- breast milk (low buffer value 6 mmol·l-1·pH-1)
- low secretion rate of acid (0.03 - 0.5 mequi/h) and pepsinogen
- retarded milk clotting
- flat postprandial reacidification
- no postprandial pepsin activation
- no pH difference between lumen and mucus
→ no guiding gradient for H. pylori
Young children:
- all diets
- small secretion rate of acid (1-5 mequi/h) and pepsinogen
- low postprandial pepsin activity
- slow postprandial reacidification
- large pH difference between lumen and mucus
→ perfect guiding gradient for H. pylori
School children:
- cow milk based meal (high buffer value 20 mmol·l-1·pH-1)
- increased secretion rate of acid (5-15 mequi/h) and pepsinogen
- fast milk clotting
- retarded postprandial increase of pepsin activity
- delayed postprandial reacidification
- pH difference between lumen and mucus
→ suitable guiding gradient for H. pylori
School children:
- diets of low buffer value
- increased secretion rate of acid (5-15 mequi/h) and pepsinogen
- postprandial fast increase of pepsin activity
- fast postprandial reacidification
- intermediate pH difference between lumen and mucus
Adults:
- all diets
- high secretion rate of acid (30 mequi/h) and pepsinogen
- postprandial fast increase of pepsin activity
- fast postprandial reacidification
- intermediate pH difference between lumen and mucus
Birth
1
2
3
4
5
6
7
8
9
10
20
30
Age in years

Supplemental Table S1
Numbers of motile *H. pylori* / nl in stomach lumen during *in vivo* experiments, expressed as mean values ± standard deviation (SD), *P* values were calculated by Student's *t* test.

Time (min)	0	2,5	5	7,5	10	12,5	15	17,5	20	22,5	25	27,5	30	*P* value 0 min versus 15 min	*n*
Profile B "Baby"	497 ± 183	381 ± 246	396 ± 404	294 ± 258	258 ± 175	195 ± 182	192 ± 187	213 ± 297	147 ± 168	91 ± 117	216 ± 323	93 ± 125	60 ± 92	0.1	3
Profile C "Young Child"	378 ± 61	196 ± 34	131 ± 58	71 ± 11	49 ± 48	13 ± 18	60 ± 66							<0.001	5
Profile C "Young Child" plus pepsin	441 ± 247	334 ± 279	188 ± 106	177 ± 213	159 ± 194	111 ± 121	106 ± 80							0.04	4
Profile A "Adult"	467 ± 30	294 ± 75	63 ± 40	142 ± 115	157 ± 70	123 ± 143	85 ± 74							0.001	3
Profile A "Adult" plus pepsin	278 ± 70	226 ± 101	162 ± 99	47 ± 25	25 ± 16	13 ± 25	0 ± 1							<0.001	4