BACTERIAL PATHOGENICITY

Intestinal colonisation of gnotobiotic pigs by *Salmonella* organisms: interaction between isogenic and unrelated strains

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The effect of intestinal colonisation by a *Salmonella* strain on the establishment in the gut of an isogenic mutant administered orally 24 h after the first strain was studied in gnotobiotic pigs. Irrespective of the clinical outcome of the infection, the extensive colonisation of one *Salmonella* strain prevented a similar degree of colonisation by an otherwise isogenic antibiotic resistant strain; in some cases the second strain was hardly detectable. The poor colonisation of the challenge *Salmonella* strains was generally reflected in very low counts of organisms in the tissues. Colonisation by a strain of *Escherichia coli* reduced the rate of establishment of an isogenic *E. coli*, strain but did not prevent colonisation by an *S. Typhimurium* strain. *S. Typhimurium* with mutations in the *tsr* (serine chemotaxis receptor protein) or *oxrA* (transcriptional regulator of anaerobic metabolism) genes did not inhibit colonisation. Mutations in *cya* (adenylate cyclase), *tar* and *trg* (chemotaxis receptor proteins for aspartate and ribose respectively) genes were less inhibitory, while *motB* (non-motile) and *cheR* (impaired motility) mutants were fully inhibitory.

Introduction

Although poultry are currently regarded as the main source of salmonella infections for man in the developed world, pigs have also traditionally been regarded as a major reservoir of zoonotic infection [1, 2].

As with poultry, salmonellosis in pigs is of both animal and public health significance. Most disease in pigs caused by *Salmonella* occurs in the post-weaning period although an uncommon severe systemic infection in neonatal pigs has been described [3]. The relatively high frequency of infection but low frequency of disease in sucking pigs is attributed to the protective effects of maternal antibody. In North America and in many developing countries, *Salmonella* serotype Choleraesuis is the most frequently isolated serotype [4] and is responsible for a septicaemia which is probably similar to typhoid in man [3, 5]. However, *S. Typhimurium* is probably responsible for most zoonotic infections from pigs. Other serotypes such as *S. Derby*, *S. Bredeney*, *S. Brandenburg* and *S. Bovismorbificans*, which produce largely asymptomatic infections in swine [3], have also been implicated in human disease.

Because of the complex epidemiology of salmonella infections, control must, of necessity, be multi-factorial [4, 6–8]. With increasing problems associated with extensive antibiotic use and abuse, and the high cost of upgrading housing to reduce environmental problems, it is likely that vaccination with live, attenuated vaccines will be increasingly used both in poultry [9, 10] and in pigs [11, 12]. In chickens, live vaccines induce immunity [13–15] and, if given orally to newly-hatched chickens, confer, within a few hours of administration, a high level of resistance to intestinal colonisation by subsequently inoculated field strains [16, 17]. This exclusion caused by the extensive colonisation of the alimentary tract by the salmonella vaccine occurs after oral infection of young animals [17–20]. This resistance to colonisation is neither the result of a rapid host immune response, nor the result of bacteriophage or bacteriocin activity [16]. It is thought to be similar to the genus-specific down regulation of bacterial growth that occurs in early stationary phase nutrient broth cultures of *Salmonella* and other enteric bacteria [21]. Therefore, live vaccines administered orally to newly hatched chickens could
confer resistance to infection within a few hours by this mechanism and would also continue to colonise sufficiently to stimulate immunity.

Because of the inherent value of such an approach to the use of live vaccines against salmonellosis in poultry and the desirability of obtaining early protection in pigs, and even more so in calves, experiments were carried out to determine whether similar specific interactions occurred between Salmonella organisms in the intestines of young animals reared normally on a whole milk diet. Such a study could also contribute to understanding of the mechanisms of intestinal colonisation of young animals by Salmonella organisms by characterising the interaction between related and unrelated bacteria when present in the gut at high density. Gnotobiotic animals were used in these initial experiments to avoid any variability in the extent of colonisation or in other factors that might occur because of different levels of acquired maternal antibody. Pigs were used rather than calves for logistic reasons. Because of the high cost of these experiments, relatively small group sizes were used in some cases; however, the results of quantitative bacteriological analysis were remarkably consistent between individual animals.

Materials and methods

Bacterial strains

The bacterial strains used in this study are listed in Table 1 [13, 16, 18, 22–30]. Mutants of Thax-1 and F98 were produced with mutations in the oxaA or cya and cheR and motB genes. These mutations were transduced into the required strain by bacteriophage P22 [13] by standard procedures. The cya phenotype was checked for very poor growth on Luria-Bertani (LB) agar. The oxaA phenotype was checked for its inability to grow anaerobically with glycerol as sole carbon source and with fumarate as electron acceptor and by the absence of stationary phase growth inhibition in vitro using a standard method [21]. Previous work (Lovell, Rychlik and Barrow, unpublished observations) had found that mutations in oxaA abolished the growth inhibition phenotype. Mutations in cya were unstable through loss of Tn10 and, therefore, a deletion mutant was produced by positive selection for tetracycline sensitivity [31] selecting for the same small colony phenotype. This mutation also abolishes stationary phase growth inhibition (Lovell, Rychlik and Barrow, unpublished observations). However, the Te5 cya phenotype also proved to be unstable, although relatively much less so, but presumably by the occurrence of secondary, suppressor mutations. The motB transcipient was non-motile and the cheR mutant showed impaired motility through soft agar [32].

In all cases, mutants of the above strains, resistant to nalidixic acid (Nal') or spectinomycin (Spc') were used. These were selected by standard methods [22]. Neither of these mutations affected the in-vivo colonisation or colonisation inhibition in young chickens [16, 17]. In all cases, Spc' was used for challenge strains.

Unless stated otherwise, all strains were cultured for 24 h in 10 ml LB broth (Difco) and generally reached counts of (3–5) × 10^9 cfu/ml. This was also the case for the cya mutant.

Experimental animals

Gnotobiotic Large-White pigs were obtained by hysterotomy and reared in metal mesh-floored cages in positive-pressure isolators [33, 34]. They were reared at a temperature of 25°–28°C and were given increasing amounts of equal volumes of sterilised condensed milk and water mixed with a mineral supplement. They were checked for freedom from bacterial contamination by culture of a rectal swab at 4 days of age aerobically and anaerobically on blood agar and were then used at 5–7 days.

Bacterial cultures in metal screw-capped bottles were taken in and out of the isolators via a port sterilised with a peracetic acid-H_2O_2 mixture for 30 min.

Infection and quantitative bacteriological analysis

Pigs were infected orally with 1-ml volumes of bacterial culture by introducing a syringe into the back of the mouth and allowing the animal to swallow the inoculum. Where the effect of colonisation of one strain on the establishment of another was to be studied, the first strain was inoculated at a dose of 10^6 cfu and the second (challenge) strain was inoculated 1 day later at a dose of 10^7 cfu. In some cases,

Table 1. Bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype/Genotype</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium F98</td>
<td>Wild-type</td>
<td>16,18,22</td>
</tr>
<tr>
<td>S. Typhimurium Thax-1</td>
<td>Non-inducer of fluid secretion</td>
<td>23,24</td>
</tr>
<tr>
<td>S. Typhimurium Bj32</td>
<td>motB::Tn10</td>
<td>25,26</td>
</tr>
<tr>
<td>S. Typhimurium Bj14</td>
<td>cheR::Tn10</td>
<td>25,26</td>
</tr>
<tr>
<td>S. Typhimurium TN2336</td>
<td>oxaA::Tn10</td>
<td>27</td>
</tr>
<tr>
<td>S. Typhimurium P3395</td>
<td>cya::Tn10</td>
<td>28</td>
</tr>
<tr>
<td>S. Typhimurium F98 motB</td>
<td>motB::Tn10</td>
<td>This paper</td>
</tr>
<tr>
<td>S. Typhimurium F98 cheR</td>
<td>cheR::Tn10</td>
<td>This paper</td>
</tr>
<tr>
<td>S. Typhimurium Thax-1 oxaA</td>
<td>oxaA::Tn10</td>
<td>This paper</td>
</tr>
<tr>
<td>S. Typhimurium Thax Δcya</td>
<td>Δcya(Tc')</td>
<td>This paper</td>
</tr>
<tr>
<td>S. Typhimurium ST1</td>
<td>Wild-type</td>
<td>29</td>
</tr>
<tr>
<td>S. Typhimurium ST330</td>
<td>ST1,tsr</td>
<td>29</td>
</tr>
<tr>
<td>S. Typhimurium ST329</td>
<td>ST1,tsr</td>
<td>29</td>
</tr>
<tr>
<td>S. Typhimurium ST832</td>
<td>ST1,tsr</td>
<td>29</td>
</tr>
<tr>
<td>Escherichia coli P4</td>
<td>Wild-type commensal</td>
<td>30</td>
</tr>
<tr>
<td>Bacteriophage P22</td>
<td>HT,int</td>
<td>13</td>
</tr>
</tbody>
</table>
bacterial strains were also inoculated singly in which case the dose was 10^3 cfu.

Faeces samples were collected daily from the anus of each animal. In a few cases, milk was sampled from the feed trough either during or immediately after feeding. Clinical condition and temperature were monitored closely. Animals were killed for post-mortem analysis when they had reached specific humane end-points (moderate dehydration, unwilling to take further food over a 24-h period and disinclination to stand).

Post-mortem analysis was done within 15 min of death. After cleaning the skin thoroughly with alcohol, the abdomen was opened and tissue samples were taken in the order cardiac blood, kidney, spleen and liver. The small intestine was tied off with string in several places to reduce movement of contents and samples were removed in the order duodenum, jejunum, ileal lymph node, ileum, caecum, colon and stomach. In most experiments, contents only were sampled. However, in one experiment intestinal mucosal samples were taken. In this case a section of the intestine was removed and, after the removal of the contents, the mucosa was washed gently in running water to remove adherent contents, dried gently and samples collected by scraping with a scalpel blade [18].

All samples were diluted in phosphate buffered saline (PBS) and homogenised by vigorous mixing or with Griffith's tubes. The number of viable bacteria in the samples was estimated by plating serial decimal dilutions on LB agar containing spectinomycin 50 μg/ml or sodium nalidixate 20 μg/ml.

**In-vitro inhibition assays**

The ability of early stationary phase cultures of different bacterial strains made in milk or LB broth to inhibit the multiplication of other inoculated organisms was assayed by standard procedures [16, 21]. In brief, 10-ml volumes of LB broth or milk, identical to that given to the pigs, were inoculated with 10^6 cfu of the bacterial strain to be tested. This culture was incubated with shaking at 37°C for 24 h. A second strain, inoculated at 10^5 cfu and with a distinguishing antibiotic resistance marker was inoculated into this culture and the mixture was re-incubated. At selected times thereafter the numbers of both organisms were counted on LB agar containing appropriate antibiotics.

**Results**

**Experiments with wild-type Salmonella strains and E. coli**

Pigs inoculated orally with either S. Typhimurium F98 Nal' followed by F98 Spc' (animals 1–4) or F98 Spc' alone (animals 5–9) became ill within 24 h of infection. The faecal bacterial counts of the inoculated strains were high at both times of sampling (Fig. 1a, b). The counts of the challenge strain (F98 Spc') in the pigs already infected with F98 Nal' were very low for the one sampling time before the pigs had to be killed because of the severity of the illness. The disease took the form of a diarrhoea and vomiting accompanied in the later stages by inappetence and lethargy.

When these animals were killed there was a fibrinous layer over the spleen and some oedema in the submucosa and between the coils of the colon. Pigs 5–9, inoculated with only 10^2 cfu of F98 Spc', had counts in excess of 10^5 cfu/g of liver and spleen and evidence of bacteraemia with *Salmonella* present in the blood and kidneys (Table 2). High counts of this strain were present throughout the gut. In pigs 1–4, inoculated with F98 Nal' followed by F98 Spc', similarly high counts of F98 Nal' were found in the tissues and gut samples. However, very few organisms of F98 Spc' were isolated from the tissues of these pigs and the counts in the gut were much lower than those of the first strain.

Oral inoculation with *E. coli* P4 resulted in faecal counts of this strain in excess of 10^10 cfu/g and occasionally 10^11 cfu/g whether this was the Nal' mutant (Fig. 1c, d) or the Spc' mutant (Fig. 1e). These high counts had little effect on the colonisation of F98 Spc' given 1 day later (Fig. 1c). These pigs were healthy at the time they were killed, although the viable numbers of the *S. Typhimurium* strain in the intestine were high. Few *E. coli* organisms were found in the non-intestinal tissues with small numbers of F98 Spc' only from the liver and spleen (Table 2, animals 10 and 11). Very high counts of *E. coli* were present throughout the gut. The counts of F98 Spc' were as high in the terminal intestine as in the pigs given this strain only. They were somewhat lower in the stomach, duodenum and jejunum.

In contrast, pre-colonisation with *E. coli* P4 retarded the establishment of P4 Spc' (Fig. 1d) compared with the rate of colonisation of P4 Spc' in pigs given this strain alone (Fig. 1e). However, numbers of P4 Spc' in the faeces of the two pigs of between 10^4 and 10^7 cfu/g were reached (Fig. 1d). The numbers of the challenge strain stopped increasing and stabilised 3 days after challenge. When these animals were killed the numbers of the first strain in the gut were much higher than those of the challenge strain. Small numbers of the first strain were isolated from the liver and spleen. The second strain was not isolated from the tissues although the cut-off in sensitivity was 10^2 cfu/g (Table 2, animals 12 to 15).

**Experiments with S. Typhimurium strains Thax-1 and ST1 and mutants of these strains**

Because strain F98 was highly virulent in this model, strains with a reduced capacity to produce gastro-
Fig. 1. Inhibition of intestinal colonisation by isogenic and non-isogenic strains of Salmonella or E. coli in gnotobiotic pigs assessed by counting numbers of viable bacteria in faeces (a) S. Typhimurium F98 Nal' (●), S. Typhimurium F98 Spc' (○); mean and SEM from four pigs. (b) S. Typhimurium F98 Spc' (●); mean and SEM from five pigs. (c) E. coli P4 Nal' (●), S. Typhimurium F98 Spc' (○); individual values from two pigs. (d) E. coli P4 Nal' (●), E. coli P4 Spc' (○); individual values from two pigs. (e) E. coli Spc' (○). ▼ indicates day on which pigs were killed because of disease; dotted line indicates limit of sensitivity of bacterial counting method (log<sub>10</sub> 2/g of gut contents).

Enteritis infections in monkeys [24] were used to study the effects of different mutations on the interaction between bacteria in the gut. All pigs infected with these strains remained completely healthy. When given alone at a dose of 10<sup>3</sup> cfu, the faecal counts of Spc<sup>+</sup> mutant of Thax-1 were very high throughout the duration of the experiment, whereas in the presence of the Nal<sup>+</sup> mutant colonisation by the Spc<sup>+</sup> mutant was inhibited (Fig. 2a, b). The Spc<sup>+</sup> mutant of ST1 was not tested on its own because of problems of virulence, but had to be used because the mutations of interest in it were not linked to an antibiotic resistance marker and could not be transduced to an avirulent strain. In this case, the pigs were infected 24 h before with E. coli P4.
The mutants of Thax-1 and ST1 tested showed varying degrees of colonisation-inhibition for the parental strains. In these experiments only two pigs were used to test each combination of strains, but the pattern of excretion of the strains was consistent in both animals used. In the pigs colonised by either the oxrA::TnlO or cya::TnlO mutant of Thax-1 Nal' the numbers of the Spc' mutant of the parent challenge strain in the faeces increased during the course of the experiment (Fig. 2c, d). In the pigs colonised by ST1 tsr Nal', the Spc' mutant of the parent challenge strain increased rapidly and eventually equalled the faecal counts of the mutant (Fig. 3b). The tar mutant in both pigs and the trg in one animal were less inhibitory than the Nal' mutant of the parent (Fig. 3c, d).

A further experiment was performed in which two groups of two pigs each were infected with E. coli P4 followed 24 h later (as above) by mutants of S. Typhimurium F98 Nal' which were cheR::TnlO or motB::TnlO and were then challenged 24 h later with F98 Spc'. The cheR and motB mutants were fully inhibitory for the parental F98 strain (results not shown).

Analysis of contents and mucosal counts of pigs infected with inhibitory and non-inhibitory strains

A comparison of the bacterial counts in the contents and mucosa of different parts of the gut in pigs infected with non-inhibitory mutants (oxrA and cya) of the Thax-1 strain showed that mucosal counts were always lower than the contents counts (Table 3). Neither the contents nor the mucosal counts of the less-inhibitory mutants were lower than those of the inhibitory parent strain.

The viable counts of the Thax-1 Nal' and Thax-1 oxrA Nal' mutant in milk samples taken from the troughs were similar to those of the stomach samples from these pigs. The counts of the Spc' challenge strains were low in these samples.

In-vitro stationary phase growth inhibition assays in milk

The ability of Spc' mutants of S. Typhimurium strains F98, Thax-1, ST1 and E. coli P4 to multiply in 24-h cultures of Nal' mutants grown in milk or broth was tested. The Nal' mutants were either of the parental phenotype or were cheR, motB, trg, tar, tsr, oxrA or cya. The full results are not shown but representative results for interactions between derivatives of Thax-1 in milk are shown in Fig. 4. Multiplication was monitored for three days after inoculation of the Spc' challenge strain. The viable counts of the first strain in the assay whose establishment did not prevent intestinal colonisation by S. Typhimurium ST1, but which reduced its virulence for the host.
Fig. 2. Inhibition of intestinal colonisation between isogenic mutants of *S. Typhimurium* strain Thax-1 using faecal bacterial counts. (a) *S. Typhimurium* Thax-1 Nal' (●), *S. Typhimurium* Thax-1 Spc' (○); mean and SEM from four pigs. (b) *S. Typhimurium* Thax-1 Spc' (○); mean and SEM from four pigs. (c) *S. Typhimurium* Thax-1 Spc' (○); individual values from two pigs. (d) *S. Typhimurium* Thax-1 Δ cya Nal' (●), *S. Typhimurium* Thax-1 Spc' (○); values for two pigs. For other details see legend to Fig. 1.

Discussion

In these experiments oral inoculation of gnotobiotic pigs with *Salmonella* or *E. coli* strains resulted in extensive colonisation with bacterial counts in the faeces exceeding $10^{10}$ cfu/g and in the case of the *E. coli* strain, $10^{11}$ cfu/g. Colonisation by wild-type *Salmonella* strains inhibited establishment by subsequently inoculated isogenic strains over the relatively

...
Colonisation of gnotobiotic pigs by S. Typhimurium strains resulted in very high bacterial numbers in the intestine which inhibited subsequent establishment by isogenic mutants inoculated 24 h later. A less pronounced inhibition also occurred between isogenic antibiotic resistant E. coli strains whereas E. coli had no effect on the establishment of S. Typhimurium F98. This degree of specificity was also found to occur with similar organisms in conventional chicks where a relatively poor level of colonisation-inhibition was also found between E. coli strains [16]. In that case, it was attributed to the difficulty in establishing an E. coli strain in birds which, in all probability, were already completely or partially colonised naturally with E. coli. Similar incomplete inhibition between heterologous E. coli strains has also been observed in gnotobiotic mice and pigs [35–37] although, in this case, it was unclear how far the absence of complete inhibition was the result of using non-isogenic strains. Much greater inhibition was observed when isogenic mutants of a K12 strain were used in gnotobiotic mice [38]. Therefore, it seems that the degree of inhibition of E. coli may be strain-dependent as can occur with Salmonella strains [39].

Because of the short interval between oral infection with the first and second strains the inhibition effect is likely to be purely microbiological, rather than an immunological phenomenon. Because the mutants used
Table 3. The effect of intestinal colonisation of gnotobiotic pigs with *S. Typhimurium* strains Thax-1 Nal' or mutants of this strain on the colonisation and mucosal association by the spectinomycin-resistant mutant of the parent given orally 24 h later.

<table>
<thead>
<tr>
<th>Site sampled</th>
<th>Pre-colonising strain</th>
<th>Challenge strain</th>
<th>Pre-colonising strain</th>
<th>Challenge strain</th>
<th>Pre-colonising strain</th>
<th>Challenge strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thax-1 Nal'</td>
<td>Thax-1 Spc'</td>
<td>Thax-1oxrA Nal'</td>
<td>Thax-1oxrA Spc'</td>
<td>Thax-1cya Nal'</td>
<td>Thax-1cya Spc'</td>
</tr>
<tr>
<td>Stomach</td>
<td>5.82</td>
<td>&lt;2</td>
<td>5.16</td>
<td>5.19</td>
<td>6.0</td>
<td>2.78</td>
</tr>
<tr>
<td>Duodenum</td>
<td>5.73</td>
<td>&lt;2</td>
<td>4.88</td>
<td>4.57</td>
<td>5.56</td>
<td>2.48</td>
</tr>
<tr>
<td>Jejunum</td>
<td>4.64</td>
<td>&lt;2</td>
<td>4.44</td>
<td>3.55</td>
<td>3.91</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Jejunum</td>
<td>5.76</td>
<td>&lt;2</td>
<td>5.72</td>
<td>5.18</td>
<td>7.28</td>
<td>3.59</td>
</tr>
<tr>
<td>Jejunum</td>
<td>3.74</td>
<td>&lt;2</td>
<td>4.13</td>
<td>3.13</td>
<td>4.61</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Jejunum</td>
<td>6.95</td>
<td>&lt;2</td>
<td>6.37</td>
<td>6.44</td>
<td>7.69</td>
<td>3.90</td>
</tr>
<tr>
<td>Colon</td>
<td>4.61</td>
<td>&lt;2</td>
<td>4.01</td>
<td>3.49</td>
<td>4.97</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Colon</td>
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<td>2.30</td>
<td>9.44</td>
<td>8.83</td>
<td>9.11</td>
<td>7.53</td>
</tr>
<tr>
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<td>6.97</td>
<td>&lt;2</td>
<td>6.08</td>
<td>5.62</td>
<td>6.97</td>
<td>5.11</td>
</tr>
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<td>Caecum</td>
<td>9.43</td>
<td>&lt;2</td>
<td>8.49</td>
<td>8.62</td>
<td>8.81</td>
<td>6.75</td>
</tr>
<tr>
<td>Caecum</td>
<td>7.72</td>
<td>&lt;2</td>
<td>6.14</td>
<td>5.71</td>
<td>7.25</td>
<td>5.61</td>
</tr>
<tr>
<td>Milk in trough Day 6</td>
<td>5.66</td>
<td>1.69</td>
<td>5.66</td>
<td>1.69</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Milk in trough Day 7</td>
<td>5.51</td>
<td>2.00</td>
<td>5.51</td>
<td>2.00</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

For doses see footnote to Table 2.

*Geometric mean of values from two pigs.

![Fig. 4. Multiplication of *S. Typhimurium* Thax-1 Spc' in 24-h milk cultures of mutants of Thax-1 Nal'.](image)

were isogenic, the inhibition was not the result of lysogenic bacteriophage or bacteriocin activity, which has also been discounted as an explanation for the colonisation-inhibition between *Salmonella* strains observed in chickens [16].

An investigation into the basis of the phenomenon by assessing the reduction in inhibition caused by mutations in genes affecting metabolism in the environment of the gut has begun. Such genes might include those affecting the switching between aerobic and anaerobic metabolism, motility and chemotaxis. The latter could be essential in the maintenance of high numbers of bacteria in mucus lining the epithelium.

The fact that the Thax-1 strain, which is non-motile, and the *motB* and *cheR* mutants of strain F98 were fully inhibitory suggests that motility *per se* is not directly involved, although the inhibitory ability of additional non-motile *TnphoA* mutants of another strain of *S. Typhimurium* were found to be significantly reduced [40]. Therefore, the reduced inhibition of colonisation shown by the *tsr* mutant of strain ST1 was of interest. This gene is responsible for the chemotactic response to serine, alanine and glycine. However, the viable counts of this strain in the faeces were 10-fold lower than those of the parent and other strains. It is possible that this may have been a factor in the absence of inhibition produced. The fact that bacterial numbers are not the only factor is indicated by the absence of inhibition produced by the *oxrA* and *cya* mutants of Thax-1, which colonised almost as well as the parent strain. Mutations in *oxrA* [41] affect regulation of carbon metabolism under different redox conditions and are thus likely to affect a number of in-vivo characteristics. Mutations in *cya* are pleiotropic [42–46] and affect utilisation of carbon sources other than glucose but also induce avirulence in mice [28].

The involvement of *cya* and *oxrA* suggests that these particular mutants might have been less efficient at using carbon sources, other than glucose, in the gut. Even in the presence of these mutants in the gut, such compounds would have been available for utilisation by...
the parent strain allowing it to multiply. This is thought to contribute to the inability of stationary phase broth cultures of such mutants to inhibit multiplication of the parent strain [46]. This also suggests that the organisms in the large intestine, at least in this model, are present in stationary phase for much of the time.

Analysis of bacterial counts in gut contents and those associated with the mucosa indicated that the numbers of bacteria in the latter niche generally reflect their numbers in the lumen in the same way that occurs in chicken gut [18]. In the chicken, and in the present study, the number of bacteria of non-invasive strains associated with the mucosa was always considerably less than that in the lumen, supporting the contention that the lumen is the main site of primary intestinal colonisation (establishment) and that, unlike enterotoxigenic E. coli [47], extensive preferential colonisation of the mucosa does not occur. There seems little ecological advantage in this occurring either in the caecum, where semi-batch incubation conditions occur, or in the colon, where the flow rate of the chyme is relatively slow [20]. However, in both organs, as in the chicken caeca, micro-organisms embedded in the mucus must act as inoculum for fresh contents entering these organs.

Given the high faeces counts, the bacterial counts in the feeding troughs were sufficiently low to rule out the possibility that inhibition may have been occurring in vivo in the small amount of contaminated milk left between feeds, as the pigs generally drank their milk soon after being fed. The fact that the results obtained in wholly liquid phase media in vitro were very similar to those obtained in vivo suggested that the main site of interaction between the strains is in the lumen of the gut rather than at the level of the mucosa.

The practical issues that come out of these studies are potentially very exciting. The results suggest that in young animals fed milk diets the oral administration of live attenuated vaccines, which colonise sufficiently well, could induce a rapid inhibition of colonisation of pathogenic organisms by a microbiological process while remaining in the gut to stimulate the immune system in the usual way. This sort of process would depend on the chosen vaccine strain being able to inhibit a sufficiently wide variety of pathogenic field strains. Work with chickens suggests that at the moment such strains have to be selected on an empirical basis [17, 39]. This aspect of vaccine use for Salmonella in chickens is now being increasingly utilised in Europe [48], although the importance of selecting inhibitory strains from which to develop vaccines is indicated by the poor inhibitory nature of the live vaccines currently available [49].

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