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Oral vaccination with a rough attenuated mutant of *S*. Infantis increases post-wean weight gain and prevents clinical signs of salmonellosis in *S*. Typhimurium challenged pigs

*[1]Neil Foster, [1]Luke Richards, [1]John Higgins, [2]Theo Kanellos and [1]Paul Barrow.

[1]School of Veterinary Medicine and Science, University of Nottingham, Nottingham UK. [2]Zoetis, International Services, Paris, France.

*Corresponding author

Neil Foster

School of Veterinary Medicine and Science

University of Nottingham

Sutton Bonington campus

Sutton Bonington

Leicestershire

NG7 2NR

Telephone: 0115 9516433

Email: n.foster@nottingham.ac.uk

Abstract

We show that oral inoculation of 5 day old conventional piglets with a rough. attenuated *S.* Infantis 1326/28Φ^r (serogroup C1), 24h prior to oral challenge with *S.* Typhimurium 4/74 (serogroup B), resulted in significant weight gain (~10%) measured at 14 days post-weaning (38 days of age). Two days after challenge the *S.* Typhimurium induced stunting and, in some cases loss, of villi but this was prevented by pre-inoculation with the *S.* Infantis strain. The clinical signs of disease associated with *S.* Typhimurium 4/74 challenge and faecal shedding were also significantly (P <0.05) reduced by pre-inoculation with the *S.* Infantis mutant. Pre-inoculation of pigs with the *S.* Infantis mutant also increased weight gain in pigs challenged with *Escherichia coli,* whereas *Mycobacterium bovis* BCG, as an unrelated intracellular bacterium, did not protect against challenge with *S.* Typhimurium 4/74.

Key words: Salmonella, Pigs, Serogroup, Vaccine, weight gain

Introduction

Salmonellosis in pigs is a problem of global significance. In 24 EU member states, levels of infection in breeder and fattening pigs have been reported to be as high as 28% and 33% respectively (Anon, 2009). In the UK between 1996-2000 over 73 000 cases of human salmonellosis were reported and *Salmonella* was attributed to most food-borne infection-related fatalities (Adak et al., 2005). In this latter survey pork and pig products were found to be important sources of human food-borne disease, whilst an earlier survey showed that *Salmonella* serovars such as *S.* Typhimurium and *S.* Derby were isolated from the caeca of 23% of pigs slaughtered in UK abattoirs (Ivanek et al. 2004). In 1997, a USA survey of the major pig producing states reported that *Salmonella* prevalence was 38.2% (Anon, 1997). A Chinese survey reported that salmonellosis accounted for 22% of the 57 612 cases of food-borne disease reported between 1994-2005 with an associated 7.8% mortality rate although this included many sources of infection (Wang et al. 2007).

Entry into the human food chain most frequently results from disease-free faecal shedding by pigs, although enteritis both in new-borne pigs, where low colostrum levels may lead to septicaemia, and in the immediate post-weaning period where contributory factors include stresses associated with changes in diet, immune status and crowding. However, even apparently sub-clinical infections may have an economic impact in the pig industry, since reduced *Salmonella* shedding is associated with increased weight gain (Farzan and friendship, 2010). Reducing *Salmonella* in pig herds would thus prevent entry of *Salmonella* into the human food chain and should increase the productivity of the pig industry.

An additional concern is reliance on antibiotics in the treatment of infected pigs and antibiotic resistant *Salmonella* serovars have been isolated from pigs with some of these being multi-drug resistant, (Sisak et al. 2006, Rosengren et al. 2008; Gomez-Laguna et al. 2011).

Vaccination is therefore regarded as a more rational and sustainable approach to the control of *Salmonella* infection in pig herds.

The three commercial *Salmonella* vaccines available for use in pigs in the EU are Salmo Shield (Novartis), Enterisol SC-54ff (Boehringer Ingelheim) and Argus SC/ST (Intervet). All of these are live attenuated vaccines derived from *S.* Choleraesuis and currently there are no licensed vaccines against *S.* Typhimurium in the UK. One study has shown that administration of the Argus vaccine to pigs is associated with significant adverse effects but it does confer some cross-protection against *S.* Typhimurium. Enterisol SC is reported to confer greater cross-protection and is not associated with significant side effects. However, neither vaccine induced sero-conversion, to produce anti-*S.* Typhimurium IgG, which did occur following *S.* Typhimurium challenge, indicating that these vaccines do not elicit a full immunological response to *S.* Typhimurium (Husa et al. 2009).

Arguello et al. (2013) have reported that intra-muscular vaccination with a formalin-inactivated S. Typhimurium DT104 (serogroup B) reduced *Salmonella* levels in fattening pens on farms where *Salmonella* was endemic. However, this vaccine did not cross-protect again *S.* Rissen (serogroup C1) which was also present. Cross protection against different serogroups is important because simultaneous infection by different serovars and serogroups has been shown in finishing pigs (Garrido, 2014). Vaccination of pigs with Argus SC/ST (Serogroup C1/B) or MeganVac1 (Serogroup B) (Lohman Animal Health International), the latter which is licensed for use in chickens, were reported to protect significantly against infection with the homologous serogroup and reportedly gave some protection against heterologous serogroups such as *S.* Infantis (Serogroup C1) when challenged 3 weeks later (Foss et al. 2013).

In an earlier study, we showed that oral vaccination with a rough, attenuated *S.* Infantis strain 1326/28Φ^r (Serogroup C1) protected highly susceptible gnotobiotic piglets against lethal oral challenge with virulent *S.* Typhimurium F98 (Serogroup B) (Foster et al. 2003; Foster et al. 2005) when this strain was administered just 24 h after vaccination. In this case it was considered that the presence of the vaccine strain in the tissues generated a high level of non-specific immunity which clearly acted against heterologous serogroups. It is therefore feasible that new vaccines which cross protect against different serogroups (and serovars) could provide protection where challenge may occur soon after a change in circumstances which precipitates clinical disease. In addition to reduced levels of disease, productivity traits should also improve. The sets of experiments described here were designed to determine this by measuring, in pigs weaned at 5 days of age, the effect of oral vaccination with the *S.* Infantis 1326/28Φ^r strain on the weight gain, faecal shedding and disease characteristics induced following challenge 24 later with a virulent *S.* Typhimurium.

Materials and Methods

Bacterial strains.

Salmonella serovar Typhimurium strain 4/74 (SL1134) has been studied extensively and is highly virulent for pigs (Paulin et al. 2007). *S.* Infantis strain 1326/28Φ^r is a poultry strain (Barrow et al. 1994) attenuated by induction of roughness by lytic bacteriophage activity (Barrow et al. 1988; Barrow et al. 1990). In all cases, mutants of these strains were made resistant to either nalidixic acid (*S.* Typhimurium) or spectinomycin (*S.* Infantis) to facilitate enumeration (Smith and Tucker, 1980). These mutations did not affect the virulence of the strains (Barrow et al. 1990; Smith and Tucker, 1980). Both strains were cultured for 24 h in 10 ml LB broth (Difco laboratories, Detroit, MI) in a shaking incubator (150 rpm) at which time they reached densities of between $3x10^9$ to $5x10^9$ CFU/ml.

Strains of enterotoxigenic *E. coli* serotypes O141:K85a,b, 88a,b and O149:K91, K88a,c were been isolated from neonatal porcine enteritis and the verotoxigenic serotype O139:K82 from a case of porcine oedema disease. These strains were also grown in LB broth. *Mycobacterium bovis* BCG was cultured in Middlebrook 7H9 broth (Difco) for 10 days prior to inoculation.

Experimental animals

Commercial Hybrid Large white/Landrace/Duroc sows were housed on straw bedding 1-2 weeks prior to farrowing. Piglets were removed from the sow at 14 days of age and inoculated with S. Infantis and or S. Typhimurium (see below). The piglets were then maintained in experimental, or control, groups in a negative pressure animal holding room, on slatted raised floors with environmental enrichment provided. The piglets were kept in a photoperiod of 11h dark/11 h light with 1h dawn and dusk, weaned at 24 days of age and fed a commercial pig rearing diet according to age and weight (Target Foods Ltd, Whitchurch, Shropshire UK). All pigs were euthanised (by injection of 5 ml sodium pentobarbital) by 38 days of age.

Experimental protocol.

All animal experiments were performed following Home Office guidelines and with appropriate Project and Personal Licenses. All experiments consisted of 4 groups of pigs, one which remained uninfected, two which received either the *S.* Infantis or *S.* Typhimurium strain only on the day of weaning or 24 later, respectively and a fourth which received th**e** *S.* Infantis strain followed 24 h later with the *S.* Typhimurium strain. Each group contained 3-4 pigs and these were replicated on 3 separate occasions.

14 day old piglets were removed from the sow and infected orally by introducing 1-ml aliquots of dilutions of a bacterial culture into the back of the mouth using a syringe without

needle, allowing the animal to swallow the inoculum. Pigs were inoculated with 10^6 CFU of the first (protective) strain and with 10^3 CFU of the second (challenge) strain with an interval of 24 h between the strains.

The pigs were monitored for clinical conditions and rectal temperature. A daily composite clinical score was calculated from a combination of physical attributes shown in Table 1. Daily scores of clinical signs of salmonellosis were recorded for each pig and mean daily scores were then calculated for each experimental and control groups. Groups which were assessed for weight changes due to infection/vaccination were vaccinated on day 14 and challenged on day 15, weaned on day 24 and euthanised on day 38. Groups in which histological changes were assessed were in vaccinated on day 14 and challenged on day 15 and then euthanised 48h later.

Faecal samples were collected daily for the first 5 days post-infection. Microbiological examination and post-mortem removal of tissues for microbiological examination was done aseptically in the order heart blood, kidney, spleen, and liver.

Histology

A 5-cm section of the terminal ileum was removed, and the lumen was flushed with a 5% paraformaldehyde solution before the tissue was fully immersed in paraformaldehyde prior to sectioning and staining with haematoxylin and eosin. Villus length and form (pointed or stunted) were assessed and compared uninfected pigs. A pathological index (Clark and Gyles, 1987; Foster et al. 2003) was used to estimate *Salmonella*-associated intestinal pathology. A scoring system of 0 to 3 determined the extent of flattening of the plica circularis and whether epithelial cell exfoliation had occurred at the tips or at the sides of the villi

Microbiological analysis

Tissue samples were homogenized by vigorous mixing (gut contents) or by Griffith's tubes or pestle and mortar with sand (tissues). The numbers of viable bacteria in the homogenates were estimated by plating serial dilutions on Brilliant Green agar (Oxoid CM263) containing spectinomycin or sodium nalidixate (50 μg/ml).

The strains of *E. coli* and the BCG strain were not enumerated.

Results

Effects on weight

The effects on animal weight at day 14 post-weaning/infection are shown in Fig. 1. The *S.* Typhimurium 4/74-challenged pigs gained approximately 14% less weight in comparison with the uninfected control pigs. In the pigs pre-inoculated with *S.* Infantis 1326/28Φ^r followed 24h later with *S.* Typhimurium 4/74, the mean weight gain was significantly increased ($P < 0.05$) after 14 days post-weaning, and was not significantly different ($P > 0.05$) from that of the uninfected control pigs or the animals inoculated with the *S.* Infantis alone.

Clinical condition

The kinetics of the clinical scores are shown in Figure 2.

In the uninfected, control pigs the highest group mean score of clinical disease (constructed from scores of weight gain, diarrhoea and behavioural data over the 14 day post-wean period was measured as 3 one day post-weaning/infection with scores on 5/14 days, In pigs inoculated with *S.* Infantis 1326/28Φ^r only, scores did not exceed 2, manifest as a slight softening of the faeces, and were also only detected in 5/14 days. The pigs infected with the *S.* Typhimurium only reached scores of between 7 and 8 at 4 days post-weaning/infection and were detectable on12/14 days. The pigs pre-inoculated with *S.* Infantis 1326/28Φ^r, prior to

challenge with *S.* Typhimurium 4/74, showed greatly reduced severity and duration of clinical signs compared to those pigs given the S. Typhimurium only, with a maximum score of between 4 and 5 at day 4.

Histology

The villi of uninfected control pigs 48 h after challenge remained long and pointed (Fig 2A; Table2) and this remained unaltered following inoculation with *S.* Infantis 1326/28Φ^r (Fig 3B; Table 1). In contrast, infection by *S.* Typhimurium 4/74 caused significant pathology in the ileum with widespread stunting and flattening of the villi and in some cases total villus loss (Fig 3C; Table2). This pathology was not observed in pigs pre-inoculated with *S.* Infantis 1326/28Φ^r prior to *S.* Typhimurium 4/74 challenge and, in this latter case, the appearance of ileal tissue was comparable to that observed in the uninfected control pigs (Fig 3D; Table 2).

Microbiology

In pigs inoculated with *S.* Infantis 1326/28Φ^r and challenged with *S.* Typhimurium 4/74 or inoculated with *S.* Typhimurium 4/74 alone faecal shedding of the *S.* Typhimurium strain had ceased by 5 days after challenge (Fig 4A and Fig 4B respectively). However, at 2 and 3 days post-challenge significantly lower numbers ($P < 0.05$) of *S.* Typhimurium were recovered from the faeces of pigs pre-inoculated with the *S.* Infantis and on day 4 it was recovered from one pig only in this group.

Salmonella organisms were not cultured from the spleens, liver and blood of any animals (data not shown).

Heterologous protection

Figure 5 shows the effect of pre-inoculation with the *S.* Infantis strains on the weight gains of pigs challenged 24 later with a mixture of pathogenic *E. coli* strains (Fig. 5a) and on the effect of pre-inoculation with *M. bovis* BCG on challenge 24 later with *S.* Typhimurium 4/74 (Fig. 4b).

Pigs inoculated with *E. coli* only showed a significant (P <0.05) 7% reduction in weight gain compared to uninfected control animals. Although pre-inoculation with *S.* Infantis 1326/28Φ^r did raise this slightly by 2%, the difference was not significant (P >0.05), although the weight was increased to a level which was also not significantly different to the uninfected controls.

Pigs which had been inoculated with *S.* Typhimurium 4/74 only showed a significant (P<0.05) 13% drop in weight gain compared with the uninfected control animals. Pre-inoculation with BCG produced a non-significant increase by 1%. Pigs inoculated with BCG only also showed no significant difference in weight gain compared with the uninfected pigs.

Discussion

Our study has shown that oral inoculation of 14 day old piglets with an attenuated, rough derivative of an avian *S.* Infantis strain (serogroup C1) was able to cross-protect against a highly virulent *S.* Typhimurium strain (serogroup B) when this was administered just 24 h after the *S.* Infantis strain, and this had prolonged positive benefits in terms of health and weight gain up to two weeks after weaning. This is not only practically but also scientifically interesting. In most experiments exploring immunity between different *S. enterica* serovars the extent of cross-protection expressed several weeks after exposure to a single *Salmonella* serovar is limited and appears only to exist where major surface antigens are in common, such as with the O-12 lipopolysaccharide antigens shared between *S.* Typhimurium (serogroup B) and *S.* Enteritidis (serogroup D). Other studies have shown only low, or no,

heterologous protection in pigs (Farzan and Friendship, 2010; Arguello et al. 2013) and chickens (Hassan and Curtiss, 1994; Beal et al. 2006). However, Foss et al. (2013) reported that although vaccination with homologous serotypes provided better protection, *Salmonella* carriage and shedding could be reduced by vaccination with a heterologous serotype. In this latter study pigs were vaccinated with either a *S.* Typhimurium vaccine used in chickens (MeganVac 1) or a *S.* Cholereasuis vaccine used in pigs (Argus) and then challenged with either *S.* Derby or *S.* Choleraesuis three weeks later. Four weeks after challenge tissues were analysed for antibody response, the numbers of IFN-γ secreting cells and bacteriology. Interestingly, the results obtained by that study suggested that serogroup-specific activity was associated with humoral immunity but cell mediated immunity was non-serogroup specific.

The difference here was that our challenge occurred within 24h of the oral administration of the protective strain such that protective specific antibody and activated T cells would not be present. The findings take further those of Foster et al. (2003; 2005) in which an attenuated *S.* Infantis strain administered orally to 5 day-old germ-free pigs provided a profound level of protection against tissue invasion and enteritis by a challenge strain of *S.* Typhimurium administered just 24 h after the first strain whilst having no effect on intestinal colonisation by the challenge strain. In that case it was argued that the high numbers of the protective strain in the intestine induced infiltration into the intestinal mucosa by large numbers of activated neutrophils which provided protection. Something similar has been shown to occur in very young chickens by using oral administration of live attenuated *Salmonella* strains again in this case with the protective strain showing high numbers in the intestine (Bohez et al. 2007). In the present set of experiments it was well known that pigs born normally to sows quickly acquire a normal flora comprising coliforms, lactic acid bacteria and anaerobes (Smith and Jones, 1963) which would provide some resistance to colonisation by the protective vaccine strains. It was therefore important to know whether the same degree of

rapid protection could be provided by oral administration of live, attenuated *Salmonella* strains to such young conventional pigs.

Similar studies by Dlabac et al (1997), Trebichavsky et al (1997) and Splichal et al. (2005) involved pre-inoculation of germ-free piglets with rough *S.* Typhimurium and *S.* Minnesota mutants which protected against challenge with *S.* Typhimurium administered 7d later and this was correlated with increased plasma IL-8 concentrations, consistent with neutrophil involvement.

Much earlier reports by Mackaness, Collins and their colleagues show cross protection between *Listeria monocytogenes* and *Brucella abortus* in mice (Collins et al. 1966) and also between *L. monocytogenes* and *S.* Typhimurium (Blanden et al. 1966). Studies by that group suggested that macrophages from infected animals were able to kill the heterologous pathogens (Mackaness, 1964; Blanden et al. 1966; see Foster et al. 2012 for review), thus also indicating innate immune protection. In those studies early protection between antigenically unrelated intracellular bacterial pathogens could be obtained by vaccinating with live bacteria within a matter of days before challenge. A similar effect had also been seen with protection against *S.* Gallinarum infection in chickens by oral or parenteral administration of the highly protective live, attenuated 9R vaccine (Smith, 1956), in this case protection being obtained therapeutically by parenteral vaccination after oral challenge with the virulent pathogen.

In chickens the cationic peptide BT/TAMUS 2032 obtained from *Brevibacillus Texasporus*, increased intestinal neutrophilia and protected against subsequent challenge with *S.* Enteritidis (Kogut et al. 2007; Kogut et al. 2010). Neutrophil-driven protection would suggests a non-specific protection against invasive bacteria and this has been shown in the case of BT/TAMUS 2032 protecting against *E. coli* (Jiang et al., 2005) as well as the previously mentioned studies on *S.* Enteritidis.

The fact that a degree of cross protection may occur between unrelated intracellular bacterial pathogens, and that we have been able to demonstrate such a rapid protective response in the gut, suggested that the BCG vaccine may have had a similar effect on enteric salmonellosis. This was not the case and may reflect the relatively slow immune response to this pathogen **(**reviewed by Cooper, 2011)**.** What was surprising was that pre-inoculation of pigs with the *S.* Infantis strain had a significant, albeit rather limited, effect on weight loss associated with simultaneous infection by different *E.* coli serotypes at 14 days post-weaning. How this occurs is unknown although it is suspected that in addition to the protective effect induced by neutrophils, other mechanisms, such as the production of defensins by intestinal Paneth cells, may also have had an effect on extra-cellular bacterial pathogens.

Whatever the mechanism behind this is, our study shows that vaccination may provide rapid protection against weight loss during infection with a pathogenic *Salmonella*, which would infer benefits in terms of productivity. It is likely that this is due to the positive effect that the *S.* Infantis strain had in preventing intestinal pathology by increasing villus length and decreasing villus flattening, thereby increasing nutrient absorption. Similar changes in villus morphology to those we have observed in *S.* Typhimurium infected pigs, were also reported in neonatal pigs fed *E.* coli LPS and in this latter study these changes were reversed by supplementary feeding with arginine (Lui et al. 2008). It is, therefore, possible that pre-inoculation of piglets with the protective *S.* Infantis strain has an indirect effect on metabolism by stimulating immune responses which inhibit infection by *S.* Typhimurium 4/74 and subsequent loss of arginine or other amino acids and/or nutrients.

Our study also suggests that pre-inoculation with *S.* Infantis 1326/28Φ^r may have some effect on S. Typhimurium carriage, including a reduction in the numbers of *S.* Typhimurium shed in the faeces of piglets and also the duration of shedding. This was not seen in the earlier studies using germ-free pig but these latter animals are far more susceptible to intestinal colonisation

and a small reduction may not have been visible. Numerous risk factors are known to influence the duration of *Salmonella* shedding in naturally infected finishing pigs. These include differences according to *Salmonella* serovar and also the age and overall health status of the pigs (Pires et al. 2014). Pires et al. (2013) also studied 18 cohorts of pigs, naturally infected with *Salmonella*, on a farrow-to-finish production system. In this study, the authors reported that there was also a cohort effect when measuring the duration of shedding and that shedding was intermittent. Although, in our study, pigs were infected directly by oral inoculation with *S.* Typhimurium, the infectious dose we administered (1×10^3) was very low. This was deliberate to attempt to introduce a more realistic approach to infection. That colonisation was detectable at all was therefore interesting. In addition, the occurrence of pathological changes in the intestine associated with salmonellosis produced by such small numbers of pathogenic bacteria was also surprising and suggests a different paradigm for enteric disease than that which is normally associated with experimental administration of relatively high numbers of bacteria. This would be worth further investigation.

In accordance with these results, a previous study has also reported that shedding dynamics are associated with clinical signs and that persistent shedders have pyrexia, tend to have diarrhoea and reduced daily weight gain (Knetter et al. 2015). Gradassi et al. (2013) have shown that oral inoculation of pigs with an attenuated S. Typhimurium 14028, which is unable to synthesise the zinc transporter ZnuABC, protects against challenge with the parent strain, administered 34 days later. In that study, pre-inoculation decreased pyrexia, diarrhoea scores and serum TNFα concentration. However, we found no difference in the expression of TNFα mRNA between any experimental or control group in the small intestine, mesenteric lymph nodes or spleen, 2 days post-vaccination/challenge (data not shown). Our results are, therefore, promising but a much larger study, which takes in to account multi-variant effects, needs to be done before we can be more confident that pre-inoculation with *S.* Infantis

1326/28Φ^{r} can inhibit or prevent shedding of *S*. Typhimurium in the immediate post-weaning period under conditions similar to those in the field. However, one study has suggested that the greatest risk factor for infecting piglets is the prevalence of *Salmonella* in the breeding herd and that the likelihood of transmission is high if the breeding herd prevalence is >10% (Hill et al. 2015). It is therefore possible that oral inoculation with the candidate vaccine strain *S*. Infantis 1326/28Φ^{r} could also be applied to the breeding herd to reduce *Salmonella* carriage in pregnant sows.

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Legends to Tables and Figures

Table 1. Disease score indices.

Daily scores of clinical signs of salmonellosis were recorded for each pig and mean daily scores were then calculated for each experimental and control groups. Four possible degrees of severity (0-3) were assessed for each clinical sign monitored (max 8 signs).

Table 2. Pathological indices for porcine intestine.

The histological picture 48h after infection employed a scoring system as follows: 0, no flattening of plica and long slender villi with no exfoliation of tips or sides of villi; 1, flattened plica with stunted villi; 2, flattened plica with stunted villi with exfoliation of tips; 3, flattened plica with stunted villi and exfoliation of tips and sides of villi. Plus/minus = Presence/absence.

Figure 1. The effect of pre-inoculation with S. Infantis 1326/28 Φ^r on weight gain of pigs challeneged 24 h later with S. Typhimurium 4/74.

Each bar shows the mean weights of 3 pigs at fourteen days post-weaning on 3 separate occasions (9 pigs in total per group). Standard deviation from the mean is shown in relation to each bar. * = significant differences in weight compared to uninfected (control) pigs at the 5% confidence limit (P = 0.05).

Figure 2. Mean clinical disease scores during 14 days observation post-infection.

Each mean is calculated from scores obtained from three pigs per group examined on three separate occasions (nine pigs per group in total).

Figure 3. Histological changes in the ileum 48 h after challenge.

Images A-C showing transverse sections of the terminal ileum of piglets stained with Haematoxylin and Eosin. (A) = Uninfected (control) intestine; (B) = Pigs inoculated with *S.* Infantis 1326/28Φ^r; (C) = Pigs inoculated with *S.* Typhimurium, (D) = 48h post-infection with *S.* Typhimurium 4/74 following pre-inoculation with *S.* Infantis 1326/28Φ^r 24h prior to challenge. Scale bar = 100 μm. Each H&E section is representative of observations obtained from 9 pigs in each experimental group.

Figure 4. Faecal counts of S. Typhimurium 4/74 from pigs during first 5 days post-infection.

(A) = *S.* Typhimurium 4/74 shed by 3 individual piglets which had been pre-inoculated with *S.* Infantis 1326/28Φ^r prior to inoculation with *S.* Typhimurium 4/74 24h later. (B) = *S.* Typhimurium 4/74 shed by 4 piglets inoculated only with *S.* Typhimurium. Data are mean and SD values. * Day 2 P=0.0146, Day 3 P=0.0194.

Figure 5. Effect of pre-inoculation with *S.* Infantis 1326/28Φ^r on weight gain in pigs challenged 24 h later with three pathogenic *E. coli* strains (A). Effect of pre-inoculation with *M. bovis* BCG on weight gains of pigs challenged 24 later with *S.* Typhimurium 4/74 (B).

Each bar shows the mean weights of 3 pigs at fourteen days post-weaning on 3 separate occasions (9 pigs in total per group). Standard deviation from the mean is shown in relation to each bar. + = significant differences in weight compared to uninfected (control) pigs at the 5% confidence limit (P = 0.05).

Table 1. Foster et al

Oral inoculation	Plica circularis and villus morphology	Exfoliation of enterocytes Villus tips	Villus sides	Score
4/74 only	Stunted, villus flattened or lost	+	-	2
1326/28 only	Pointed and long	-	-	0
1326/28 + 4/74	Pointed and long	-	-	0
Uninfected control	Pointed and long	-	-	0

Table 2. Foster et al

Signs	Condition scores			
	0	1	2	3
Demeanour	Normal	Isolated	Hunched,	Shuddering
Activity	Normal	Abnormal posture, dull/depressed	Inactive or overactive	Clearly ill
Gait	Normal	Minor incoordination, abnormal gait	Uncoordinated, abnormal footplant, reluctant to move	Staggering, limb dragging, paralysis
Weight	Normal (weight gained)	Lost 0-50 grams in 24 hours	Lost 50-100g in 24h	Lost 100g in 24h
Drinking	Normal	More or less than normal in 24h	More or less than normal in 48h	Constantly drinking or not drinking in 48h
Eating	Normal	Less than normal in 24h	Less than normal in 48h	No appetite
Faeces	Normal	Small amount of diarrhoea	Large amount of diarrhoea	Liquid or blood in faeces
condition	Normal	Weight loss in hind limb	Weight loss hind limb and shoulder blade	N/A

Fig 1 Foster et al

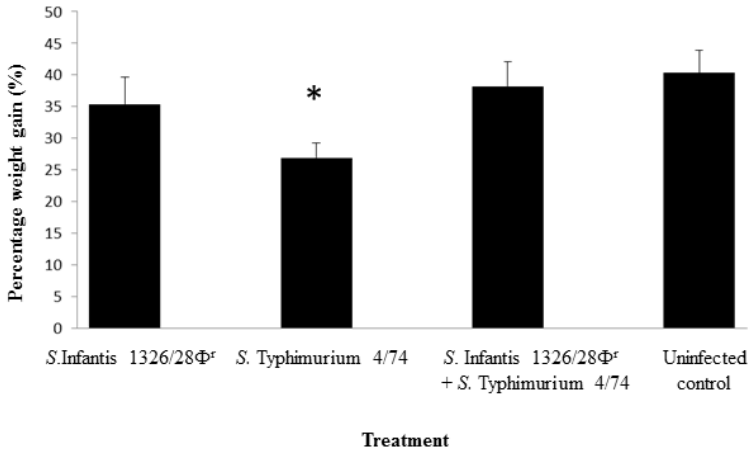


Fig 2 Foster et al

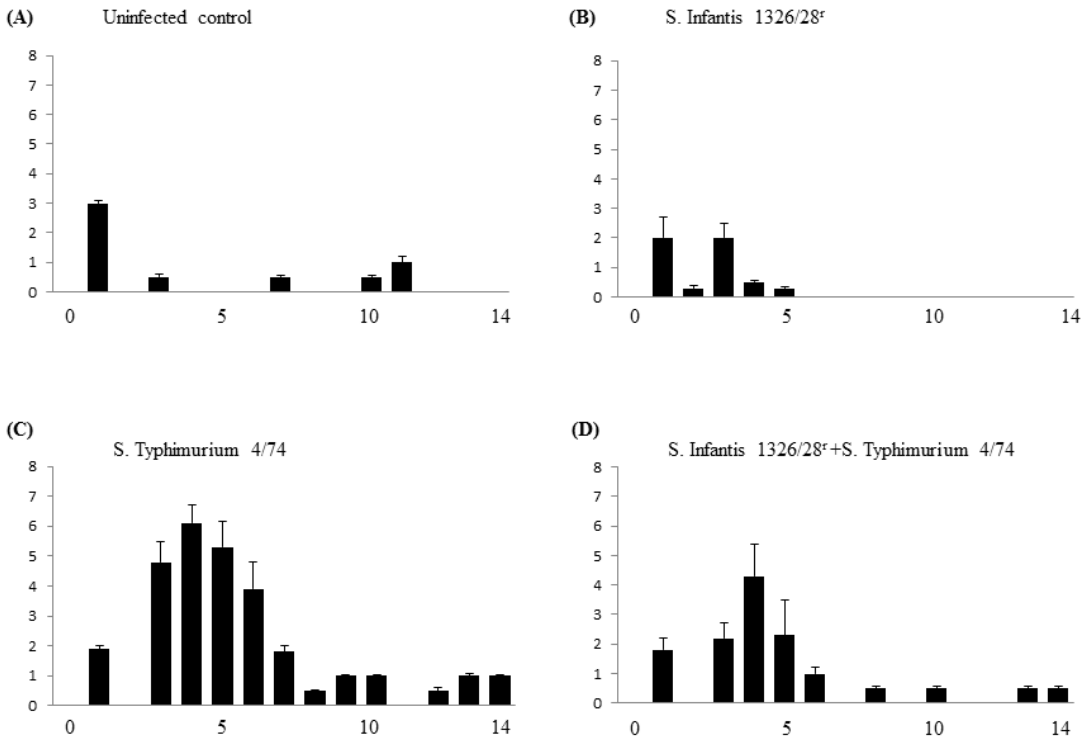
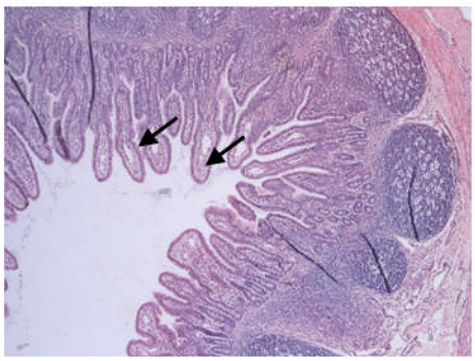
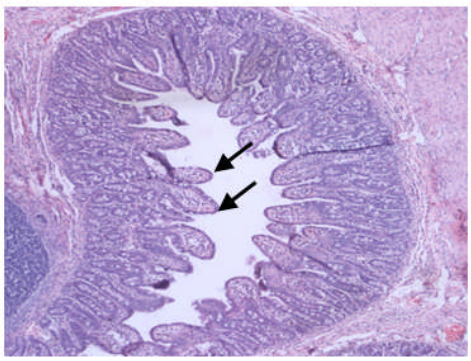


Fig 3 Foster et al

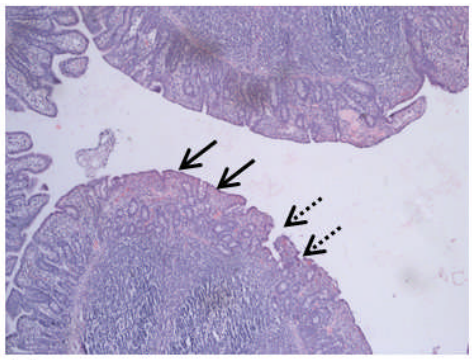
(A) Control ileal tissue



(B) *S.* Infantis only



(C) *S.* Typhimurium 4/74 only



100 µm

(D) *S.* Infantis + *S.* Typhimurium 4/74

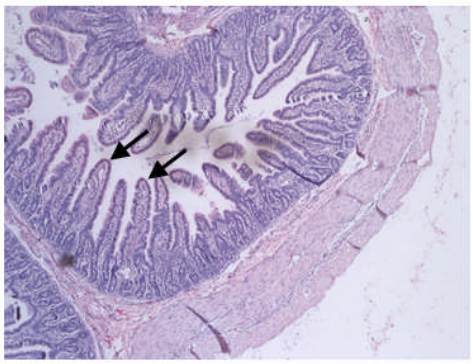


Fig 4 Foster et al

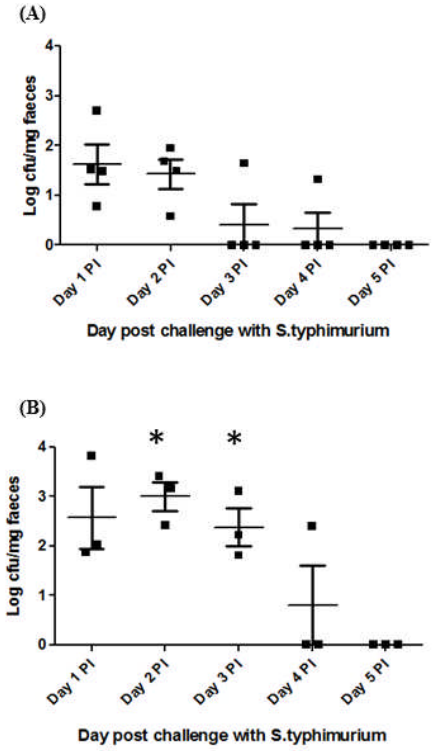


Fig 5 Foster et al