

A clinical field trial to evaluate the efficacy of vaccination in controlling *Salmonella* infection and the association of *Salmonella*-shedding and weight gain in pigs

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Abstract

A clinical field trial was performed to determine the effectiveness of an autogenous *Salmonella* Typhimurium bacterin compared with a commercial live *S. Choleraesuis* vaccine in pigs. The association between *Salmonella* shedding and weight gain was also investigated. Nine cohorts of weaned pigs, (330 to 350 pigs per cohort), were randomly assigned to 1 of 3 treatment groups (injection with *S. Typhimurium* bacterin, vaccination via water with *S. Choleraesuis* vaccine, or a control group receiving no vaccine). In each cohort, the average daily gain was calculated for a selected pen throughout the production stage. Pen (pooled) fecal samples were collected bi-weekly and cultured. The odds of *Salmonella* shedding in both vaccinated groups was higher than in the control group ($P < 0.05$). The prevalence of *Salmonella* shedding declined overall as pigs aged ($P = 0.04$). However, the control pigs showed the smallest decrease in *Salmonella* shedding over the entire production stage, while prevalence of *Salmonella* shedding in the vaccinated groups decreased twice as much as the control group over the entire production stage. *Salmonella* Typhimurium var. Copenhagen DT104, *S. Cerro*, and *S. Agona*, which had been isolated on the study farm previously, were recovered from pigs in this study. Shedding of *S. Typhimurium* var. Copenhagen decreased over time in both vaccine treatment groups. On the other hand, *S. Cerro* shedding rate was lower in the control pigs compared with vaccinated pigs and *S. Agona* could be recovered only from the samples collected from *S. Choleraesuis* vaccinated pigs. The pigs from pens with a higher *Salmonella* recovery rate experienced slower growth compared with pigs from pens where *Salmonella* was not isolated. This latter finding indicates that there might be an economic incentive for producers to try to control endemic salmonellosis if effective programs could be developed.

Résumé

Un essai clinique a été réalisé afin de comparer l'efficacité d'une bactérie autogène de *Salmonella* Typhimurium à un vaccin vivant commercial de *S. Choleraesuis*. L'association entre l'excrétion de *Salmonella* et le gain de poids a également été étudiée. Neuf cohortes de porcs sevrés (330 à 350 porcs par cohorte) ont été réparties de manière aléatoire à un des 3 groupes de traitement (injection avec la bactérine de *S. Typhimurium*, vaccination via l'eau avec un vaccin *S. Choleraesuis*, ou un groupe témoin ne recevant aucun vaccin). Dans chaque cohorte, le gain moyen quotidien a été calculé pour un enclos sélectionné durant toute la période de production. Des échantillons de fèces pris dans les parcs ont été récoltés bi-hebdomadairement et mis en culture. Les probabilités d'excrétion de *Salmonella* dans les deux groupes vaccinés étaient plus élevées que dans le groupe témoin ($P < 0,05$). La prévalence d'excrétion de *Salmonella* a diminué à mesure que les porcs vieillissaient ($P = 0,04$). Toutefois, les porcs témoins ont présenté la plus petite réduction dans l'excrétion de *Salmonella* durant toute la période de production, alors que la prévalence d'excrétion de *Salmonella* dans les groupes vaccinés durant toute la période de production a diminué deux fois plus que le groupe témoin. *Salmonella* Typhimurium var. Copenhagen DT104, *S. Cerro* et *S. Agona*, qui avaient été isolés préalablement sur la ferme à l'étude, ont été isolés à partir de porcs dans cette étude. L'excrétion de *S. Typhimurium* var. Copenhagen a diminué dans le temps chez les deux groupes d'animaux vaccinés. Par contre, le taux d'excrétion de *S. Cerro* était plus faible chez les porcs du groupe témoin comparativement aux porcs vaccinés et *S. Agona* n'a été retrouvé qu'à partir des échantillons prélevés des animaux vaccinés avec *S. Choleraesuis*. Les porcs provenant des enclos avec un taux plus élevé d'isolement de *Salmonella* présentaient une croissance ralentie comparativement aux porcs provenant des enclos où on ne retrouva pas de *Salmonella*. Cette dernière trouvaille indique qu'il y aurait un incitatif financier pour les producteurs à tenter de limiter la salmonellose endémique si des programmes efficaces pouvaient être développés.

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Introduction

Salmonella continue to be important foodborne pathogens with a significant economic impact (1). Exposure to *Salmonella* either by direct contact with infected pigs or through consumption of contaminated pork, is an important mode of infection in humans (2). In addition to the public health issue, *Salmonella* have the potential to cause clinical disease in pigs (3) resulting in a significant economic impact on swine production.

It is necessary to take the presence of *Salmonella* on swine farms as a serious issue and apply appropriate practical intervention strategies. Immunization appears to be one of the most promising approaches for control (4–8). *Salmonella* Choleraesuis vaccines are commercially available and may provide cross-protection against other *Salmonella* serovars as well (9); however, *S. Choleraesuis* does not appear to be a common pathogen in Ontario swine herds any longer (10–11).

In contrast, *Salmonella* Typhimurium var. Copenhagen has become the most frequent serovar recovered on Ontario swine farms (11), and yet it only occasionally causes clinical disease (12). More frequently *S. Typhimurium* is present without the herdsman noticing any clinical signs (13). There is little information regarding the economic impact of *S. Typhimurium* infection in pigs, particularly in herds with subclinical infection. Although live *S. Typhimurium* vaccines have been reported to protect pigs against *Salmonella* shedding in experimental challenge studies (14–15), there are no commercial *S. Typhimurium* vaccines available for use in swine.

The objectives of the present study were i) to determine if an autogenous *S. Typhimurium* var. Copenhagen vaccine or a commercial live *S. Choleraesuis* vaccine can reduce the prevalence of *Salmonella* shedding in market weight hogs under field conditions, ii) to investigate whether or not *Salmonella* vaccination can improve growth performance, and iii) to determine if *Salmonella* shedding affects weight gain in pigs.

Materials and methods

Study design and sample collection

The trial was conducted on one farrow-to-finish pig operation with a history of clinical and sub-clinical salmonellosis. Previously, *Salmonella* Typhimurium var. Copenhagen DT104 and *Salmonella* Cerro had been recovered from fecal samples collected from grower-finisher pens on this operation (10–11). In addition, *Salmonella* Agona had been isolated from specimens from clinical cases of salmonellosis on this farm (Farzan and Friendship, unpublished data). The biosecurity measures on this farm included, but were not limited to, shower-in/shower-out for visitors and farm workers, all-in/all-out management of rooms with washing and disinfection between fills, and single-source supply of replacement gilts.

Nine cohorts of weaned pigs, approximately 350 pigs in each group, were randomly assigned to 1 of 3 treatment groups. Group 1—intramuscular injection with an autogenous *S. Typhimurium* bacterin (4 cohorts); group 2—in-water administration of a modified-live *S. Choleraesuis* vaccine (Argus SC/ST, Intervet CanadaCorp, Kirkland, Quebec) (3 cohorts); group 3—an

untreated control group (3 cohorts). In each cohort, the weaned pigs were housed in a 4-pen room (stage-1 nursery) for 2 to 3 wk, after which the pigs were moved into a 6-pen room (stage-2 nursery). In each cohort, one pen was randomly selected in the nursery stage and 30 pigs were ear-tagged and weighed. After 4 to 7 wk, the pigs were moved into 12-pen rooms (grower-finisher stage) and kept there until marketing. The tagged pigs were weighed again when marketing. Pooled-fecal samples were collected from manure found on the pen floor prior to vaccination, weekly from each nursery pen, and bi-weekly from grower-finisher pens.

Salmonella identification

The presence of *Salmonella* in the fecal samples was tested at the Laboratory Service Division, University of Guelph according to MFHPB-20 (16) and MFLP-84 (17) protocol. Briefly, a 10% suspension of fecal samples in 0.85% saline was prepared and 1 mL transferred into 10 mL of Buffer Peptone Water (BPW). The pre-enrichment mixtures were incubated at 35°C for 18 to 24 h, then 1 mL of culture was reacted with 20 µL of anti-*Salmonella* beads (Dynabeads; Invitrogen Corporation, Carlsbad, California, USA). The beads were washed twice in 1 mL of PBST (phosphate buffered saline containing 0.01% Tween 20, pH 7.4), and concentrated in 100 µL of PBST using an automated immunomagnetic separator (Invitrogen Corporation). The 100 µL concentrates were transferred into 10 mL of Rappaport-Vassiliadis Soya Peptone Broth (RVS) (Oxoid, Nepean, Ontario) and incubated at 42 ± 0.5°C for 18 to 24 h. A loopful from the RVS cultures was plated on brilliant green sulfapyridine (BGS) and bismuth sulfite (BS) agars and incubated for 24 ± 2 h at 35 ± 0.5°C. Confirmation of *Salmonella* isolates was performed by serological and biochemical means using triple sugar iron agar (TSI), lysine iron agar (LIA), urea slants, and *Salmonella* O antiserum Poly A-I and Vi slide agglutination tests (Difco, Detroit, Michigan, USA). The isolates were submitted to the Reference Laboratory for Salmonellosis, Laboratory for Foodborne Zoonoses (LFZ), Public Health Agency of Canada, Guelph for serotyping and phage typing.

Preparation of autogenous vaccine

The autogenous vaccine was prepared at Gallant Custom Laboratories, Cambridge, Ontario using a *S. Typhimurium* var. Copenhagen DT104 strain previously isolated from the study farm (11). Briefly, 1000 mL of trypticase soy broth (TSB) was inoculated with a single colony of the *S. Typhimurium* var. Copenhagen DT104 and incubated at 37°C aerobically for 6 h using agitation. In order to determine the number of colony forming units (CFU/mL) serial dilutions of bacterial cultures in isotonic saline (0.9% NaCl) buffer were prepared and 0.1 mL of diluted cultures were plated on XLT-4 plates and incubated at 37°C overnight. The 1000 mL TSB culture was centrifuged and the pellet was washed with PBS buffer containing 0.45% formalin. The wash and centrifugation steps were repeated 5 times. The bacterial cells were suspended in alhydrogel in a ratio 1:3 (antigen: adjuvant) to obtain 10⁸ CFU/mL bacterin. The vaccine sterility was controlled by plating on blood agar and *Salmonella* selective media. To test the safety of the vaccine, a dose of 2 mL was intramuscularly injected into 2 weaned pigs and the pigs were observed for 1 wk.

Data analysis

Data were entered into a spreadsheet (Microsoft Excel 2003; Microsoft, Redmond, Washington, USA) and imported into statistics software (Stata 9.1 Intercooled for Windows XP; StataCorp, College Station, Texas, USA). A Generalized Linear Latent and Mixed Models (GLLAMM) was used to compare the presence of *Salmonella*, as well as prevalence of most frequent serotypes and phagetypes in the pooled fecal samples collected from the pens in 3 groups. A repeated measurement index was included as random effect into the model to take the correlation between weekly and bi-weekly testing into account. A mixed linear regression method with pen as random effect was applied to compare the average daily gain in the vaccinated and the control pigs. Since the average starting weight of pigs did differ in 3 cohorts, it was included in the model. Sex and age of pigs were considered for initial inclusion in the regression analysis. In order to investigate the impact of *Salmonella* shedding regardless of vaccination, the average daily gain was regressed versus the recovery rate of *Salmonella* from the pens where the ear-tagged pigs were kept. The presence of confounding and interaction, between weight of pigs at weaning and age of pigs at sampling, with the main effect was investigated in each model.

Results

Salmonella shedding

In total, 653 pooled pen samples were collected from stage-1 nursery (20 samples), stage-2 nursery (201 samples), and finisher (432) rooms. *Salmonella* were cultured from 85 (13%) of the samples. The number of *Salmonella*-positive samples in each group is shown in Table I. The *Salmonella* recovery rate differed among the 3 groups before and after vaccination with the lowest in the control group (5.9%) and the highest in the *S. Choleraesuis* vaccinated group (19.0%). In the multilevel analysis, the odds of *Salmonella* shedding in both vaccinated groups was higher than in the control group (Table II). The *S. Choleraesuis* vaccinated pigs were more likely [OR = 5.2 (1.8, 15.5)] to shed *Salmonella* compared with control pigs. Similarly the odds of *Salmonella* shedding was 3.3 (1.1, 9.9) greater in the pigs that were injected with autogenous vaccine versus control pigs. In multivariable analysis while controlling for vaccination, the prevalence of *Salmonella* shedding declined overall when pigs aged ($P = 0.04$). Overall, the recovery of *Salmonella* decreased from 24.5% at weaning to 10.0% at marketing (Figure 1, Table II). The control pigs showed the smallest decrease (8.0%) in *Salmonella* shedding over the entire production stage with decreasing from 8.8% at weaning to 0.8% at marketing age (Figure 1). In contrast, prevalence of *Salmonella* shedding in the vaccinated groups decreased twice as much as the control group over the entire production stage. Prevalence of *Salmonella* shedding in the *S. Typhimurium* vaccinated group declined from 24% prior to vaccination to 8.5% in marketing-age pigs (Figure 1). *Salmonella* shedding among the *S. Choleraesuis* vaccinated pigs declined from 32.0% prior to vaccination to 16.0% at marketing age (Figure 1).

Salmonella serovars

Nine different *Salmonella* serovars were identified over the entire period of the trial. *S. Cerro* was the most frequent serovar isolated followed by *S. Typhimurium* var. Copenhagen (Figure 2). *Salmonella* Typhimurium var. Copenhagen was isolated from 72.7% of samples collected from unvaccinated pigs, while it could only be cultured from 26.8% and 32.3% of samples collected from oral and autogenous vaccinated pigs, respectively (Figure 2). On the other hand, *S. Cerro* shedding rate was lower in the control pigs compared with vaccinated pigs in that it was isolated from 27.3% of samples in the control pigs versus 48.8% and 51.6% of samples collected from pigs that received oral live *S. Choleraesuis*, and injectable autogenous killed vaccine, respectively. *Salmonella* Agona could be recovered only from the samples collected from *S. Choleraesuis* vaccinated pigs. *Salmonella* Typhimurium var. Copenhagen phagetypes isolated from pigs in 3 groups are shown in Figure 3. Three phagetypes were identified among the 31 *S. Typhimurium* isolates including DT104b (1 isolate), U311 (6 isolates), and Untypable (UT) (24 isolates). No DT104 phagetypes were identified and the only 1 DT104b isolate was recovered from the control pigs.

Average daily weight gain

In total, 251 pigs were ear-tagged in the 9 cohorts and housed in separate pens during the nursery and finishing stages. *Salmonella* was cultured from 5 (10.4%), 7 (13.5%), and 2 (6.5%) of the samples from pens housing *S. Choleraesuis* vaccinated, *S. Typhimurium* vaccinated, and non-vaccinated controls, respectively. The average daily gain in these 3 groups was determined as 736.5 g, 647.9 g, and 762.6 g for *S. Choleraesuis* vaccinated, *S. Typhimurium* vaccinated, and non-vaccinated controls, respectively. In the multilevel analysis, the control pigs that had the lowest average of *Salmonella* shedding showed best growth performance compared with the vaccinated groups (Table III). In order to investigate the impact of *Salmonella* shedding regardless of vaccination, the average daily gain was regressed versus the recovery rate of *Salmonella* from the pens where the ear-tagged pigs were kept. The multilevel analysis showed that the pigs from pens with a higher *Salmonella* recovery rate were deemed to have a lower average daily gain ($P = 0.059$) (Table IV).

Discussion

Salmonella Typhimurium var. Copenhagen has become the most frequent serovar on Ontario swine farms (10–11). There is no *S. Typhimurium* vaccine registered for use in pigs in Canada but *S. Choleraesuis* vaccines are commercially available. *Salmonella* Choleraesuis modified live vaccine was reported to cross-protect against other *Salmonella* serovars (9). Husa et al (5) have recently conducted a controlled challenge trial on 2 different modified live *S. Choleraesuis* vaccines, including Argus (SC/ST, Intervet) and Enterisol SC-54 (Boehringer Ingelheim Vetmedica, St. Joseph, Missouri, USA). They have shown that the enteric lesion and clinical scores in the pigs vaccinated with Argus were similar to the control pigs and that the vaccine could not reduce *Salmonella* shedding after the animals were challenged with *S. Typhimurium*. In addition, the growth rate was lower in the Argus-vaccinated pigs compared with controls in the Husa et al (5) trial. Yet, the effectiveness of the

Table I. Culture of *Salmonella* from pooled manure samples collected from pens housing pigs assigned to 1 of 3 treatments: *S. Choleraesuis* live oral vaccine, *S. Typhimurium* autogenous bacterin given intramuscularly, and non-vaccinated controls

Treatment group	Number of positive samples/total (%)			
	Nursery 1	Nursery 2	Finisher	Total
<i>S. Choleraesuis</i> vaccine	Not sampled	14/75 (18.7)	30/156 (19.2)	44/231(19.0)
<i>S. Typhimurium</i> vaccine	4/20 (20.0)	8/78 (10.3)	19/156 (12.2)	31/254 (12.2)
Control	Not sampled	4/48 (8.3)	6/120 (5.0)	10/168 (5.9)
Total	4/20 (20.0)	26/201 (12.9)	55/432 (12.7)	85/653 (13.0)

Table II. The impact of vaccination and age of pigs on *Salmonella* shedding in the vaccinated and control pigs analyzed by Generalized Linear Latent and Mixed Models

	Odds ratio	Standard error	95% Confidence interval	P-value
<i>S. Choleraesuis</i> vaccine	5.2	2.9	1.8, 15.5	0.003
<i>S. Typhimurium</i> vaccine	3.3	1.8	1.1, 9.9	0.037
Control Reference				
Age (week)	0.9	0.0	0.8, 0.99	0.04

Table III. The effect of *Salmonella*-vaccination on average daily weight gain in pigs

Group	Coefficient (g)	Standard error	95% Confidence interval	P-value
<i>S. Choleraesuis</i> vaccine	-26.6	13.5	-53.0, 0.27	0.048
<i>S. Typhimurium</i> vaccine	-90.8	16.2	-122.5, -59.0	< 0.001
Control Reference				
Weight at weaning (kg)	4.7	2.1	0.7, 8.7	0.022
Intercept	694.5	31.2	633.3, 755.8	< 0.001

Table IV. The effect of *Salmonella* shedding on average daily weight gain in pigs

Parameter	Coefficient (g)	Standard error	95% Confidence interval	P-value
<i>Salmonella</i> -positive samples (%) ^a	-3.0	1.6	-6.1, 0.4	0.059
Weight at weaning (kg)	10.5	1.8	7.0, 14.0	< 0.001
Intercept	608.3	34.5	540.7, 675.9	< 0.001

^a Percent of *Salmonella*-positive samples collected from pens where the ear-tagged pigs were kept.

S. Choleraesuis vaccine and its protection against other serovars under farm conditions remains unknown. Nevertheless, because of the significant labor required to inject vaccines into nursery pigs, compliance may be a problem in the case of autogenous bacterins and *S. Choleraesuis* modified-live vaccine may be preferred due to being easier to administer. The primary goal of the present study was to investigate the effectiveness of a commercial oral live *S. Choleraesuis* vaccine compared with an autogenous *S. Typhimurium* bacterin to control *Salmonella* shedding in the market weight pigs under field conditions. Neither vaccination approach proved to be better than non-vaccinated controls to reduce *Salmonella* sp. shedding. However, as this was a field trial, there were factors that could not be easily controlled for and as such, interpretation of the data was difficult. The prevalence of *Salmonella* shedding was different among the treatment groups prior to vaccination in that it was the lowest in the control group. Vaccination was associated with a significant decrease in shedding from nursery to market; however, this could have been because the level was high to begin with. It has previously been shown that younger pigs are more likely to shed *Salmonella* than finisher pigs (18); therefore, our estimate of vaccine effectiveness may be biased because the decrease in shedding might

be due to age. In order to take this into account "age of pig at the sampling date" was included into the multivariable analysis.

The vaccination was likely done after pigs had been exposed and that efficacy might have been improved had the pigs been vaccinated sooner. At least one study suggests that it might be prudent to establish a vaccination strategy for pregnant sows to control *Salmonella* in their piglets (19) followed by another vaccination at weaning age.

Salmonella vaccination strategy using the live attenuated strains administered orally has been suggested as the most effective approach (20), as oral immunization can stimulate local gut immunity (21) inducing mucosal IgA secretion in the gut (22) that is important in protection. The oral *Salmonella* live vaccines can also induce cell-mediated immunity that may play a major role in protecting pigs because *Salmonella* are facultative intracellular pathogens and may avoid humoral immune response in the intracellular environment (23). However, despite a lack of protection for pigs against *Salmonella* shedding, the *S. Choleraesuis* vaccine proved to be easily administered in the drinking water, and this approach was readily acceptable by the producer.

Salmonella Typhimurium var. Copenhagen DT104, *S. Cerro*, and *S. Agona*, which had been previously isolated on the study farm

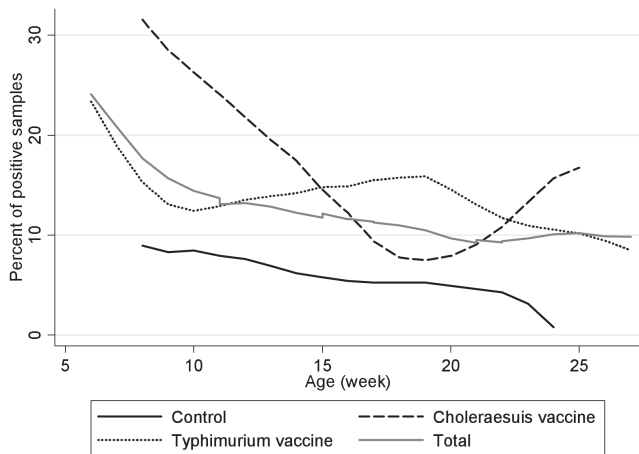


Figure 1. *Salmonella* shedding trends among vaccinated and control pigs over the entire production stage.

(10–11), could be recovered from pigs in this study. Although neither vaccination approach proved to be better than non-vaccinated controls to reduce *Salmonella* sp. shedding, both vaccines were associated with a decline in shedding of *S. Typhimurium* var. Copenhagen. The failure of the *S. Choleraesuis* vaccine to reduce *Salmonella* Agona shedding, however, might be due to lack of cross-protection. Although *S. Choleraesuis* vaccine was reported to cross-protect against *S. Typhimurium* (9), it did not protect pigs against shedding other members of serogroup B (*S. Agona*) in the present study. *Salmonella* Cerro, a member of serogroup K with somatic antigen O18, was the most frequent serovar recovered from both vaccinated groups suggesting that neither *S. Typhimurium* (serogroup B; antigen O4) nor *S. Choleraesuis* (serogroup C1; antigen O6, 7) cross-protect the pigs against a member of serogroup K, such as *S. Cerro* with somatic antigen O18. On the other hand, the pigs vaccinated with *S. Typhimurium* did not appear to shed *Salmonella* Agona and since *S. Typhimurium* and *S. Agona* are members of serogroup B carrying O4, cross-protection between these serovars might be effective.

These findings indicate that the most logical approach to *Salmonella* vaccination would be to try to develop a multivalent *Salmonella* vaccine containing multi-serogroup somatic antigens that could be administered in the drinking water. In addition to making the product easy to use, it is also likely that oral immunization could induce local gut immunity and cell-mediated immunity.

Another significant finding of this study is that pigs that appeared clinically healthy but were found to be shedding *Salmonella* grew slower than pigs not shedding *Salmonella*. This suggests that there is an economic cost to subclinical *Salmonella* infection. Fraser et al (24) have shown that experimental infection with *Salmonella* Typhimurium did not affect the average daily gain in pigs while infection with *Salmonella* Choleraesuis reduced the average daily gain. Husa et al (5) have recently conducted an experimental trial and shown that the growth rate was lower in the Argus-vaccinated pigs compared to unvaccinated control pigs. However, to the best of our knowledge, the present study is the first report on the impact of *Salmonella* shedding on weight gain in pigs under field conditions. These findings indicate that the cost of intervention strategies might

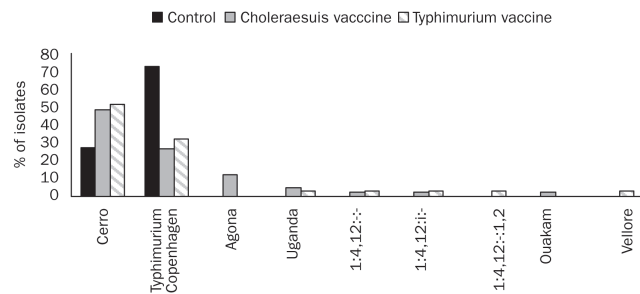


Figure 2. *Salmonella* serovars isolated from fecal samples collected from vaccinated and control pigs.

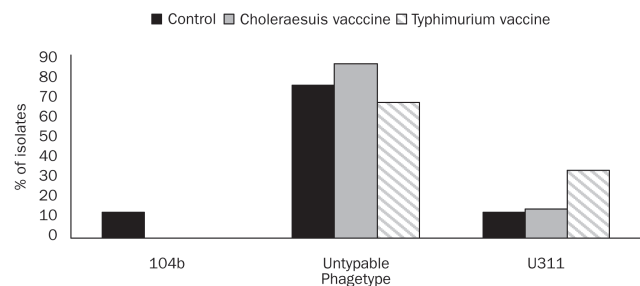


Figure 3. *Salmonella* Typhimurium var. Copenhagen phagetypes isolated from fecal samples collected from vaccinated and control pigs.

be compensated by improved weight gain in the absence of clinical disease. Therefore, there would be an economic incentive for producers to institute the control measures if a practical and effective control program could be devised.

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