



Salmonella contamination in a network of 10 pig farms interconnected within the same cooperative

Alexandra Henry, Ann Letellier, Jean-Charles Côté, Gabriel Desmarais, Virginie Lachapelle, Nadia Bergeron, Sylvette Lewandowsky, Philippe Fravalo

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ABSTRACT

Objective To evaluate whether pig farms interconnected within the same cooperative share similar *Salmonella* contamination patterns.

Setting Ten finishing pig farms within a 100 km radius of a common slaughterhouse were selected. Their inclusion was based on their association to the same cooperative and the sharing of common resources: piglets, feed, swine transporters, slaughterhouse, technicians and veterinarians.

Procedure Each farm was visited three times over a 10-month period. Pig faeces, the barn front door handle, the feed pipeline, mobile objects (shovel, balance and pig board), the landing stage, the concrete slab of the feed bins, the tire tracks left on the pathways by the animal feed truck, the pig delivery truck and the carcass knacker truck and the mudguards and cabin carpets of the veterinarian and technician vehicles on their arrival at the farm were all analysed for the presence of *Salmonella*.

Results All farms were not equally contaminated with *Salmonella*. Whereas some farms yielded up to 12 *Salmonella* isolates, other farms were *Salmonella* free. Some locations, most notably the landing stage, were more contaminated than others. *Salmonella* contamination was dynamic in time. Some contaminations seen on farms, on specific locations on the first visit, had disappeared on the second and third visits, but new contaminations were detected on different locations.

Conclusions Contamination with *Salmonella* was not disseminated through the network of the 10 pig farms interconnected within the same cooperative but was rather most often restricted in time to specific locations on specific farms.

INTRODUCTION

Salmonella is a genus of Gram-negative, rod-shaped bacteria and a member of the class γ -proteobacteria and the family *Enterobacteriaceae*. It is the causal agent of salmonellosis, a zoonotic infectious disease characterised by abdominal cramps, diarrhoea, fever and vomiting. In Western countries, salmonellosis is the second most common foodborne disease. Whereas *Salmonella enterica* subsp. *enterica* serotype Enteritidis, referred to as *S. Enteritidis*, infection, is mostly associated to consumption of raw or undercooked eggs, *S.*

enterica subspecies Typhimurium, *S. Typhimurium*, infections are more largely represented in pork, poultry, beef, dairy products and so on.¹

Salmonella is endemic in pig and carriage is mostly asymptomatic.² Domestically produced pig is the second most important animal-food source of human salmonellosis.³

Mitigation measures have been implemented at three levels: (1) at preharvest, the control of *Salmonella* in pig, (2) at harvest, through improved hygiene during slaughter and meat processing and (3) at postharvest, the preparation of food by the industry and the consumer.⁴

Several risk factors for the transmission of *Salmonella* to pig have been well studied,⁵ including the role of rodents as vectors for *Salmonella*,⁶ hygiene,⁷⁻⁸ lairage conditions,⁹⁻¹⁰ sources of animals,⁸ herd management,¹¹ production system,¹² feeds,¹³ antibiotics,¹⁴ parasite infestations¹⁵ and so on. However, none of these have focused on the comparative presence and dynamics of *Salmonella* on a network of farms interconnected within a common cooperative with shared common resources: piglets, feeds, swine transporters, a slaughterhouse, technicians and veterinarians. Here the presence of *Salmonella* is described in such a network of 10 pig farms, with three visits per farm over a 10-month period.

MATERIALS AND METHODS

Pig farms

A network of 10 finishing pig farms (A–J) was selected within a 100 km radius of their common slaughterhouse. Their inclusion was based on their association to the same cooperative that provided common resources: a breeding facility for the production of all piglets for its members, feeds, swine transporters, a slaughterhouse, and technicians and veterinarians.



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Pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint Hyacinthe, Quebec, Canada

Correspondence to

Dr Philippe Fravalo; philippe.fravalo@umontreal.ca

Sampling

Each farm was visited three times over a 10-month period, from August 2011 to May 2012. These three visits were done during two consecutive production batches and at the end of the growing period of the second batch, respectively. Inside the barn, five different pools of approximately 100 g of fresh faeces, each from three separate pens, were collected. Additional samples were obtained by swabbing four different locations or series of objects: the barn front door handle and a 20 cm × 20 cm area around it on both sides of the door, a 100 cm section of the feed pipeline, mobile objects (shovel, balance and pig board) and a 30 cm × 30 cm surface of the landing stage. Likewise, outside the farm, samples were collected by swabbing a 30 cm × 30 cm surface from four locations: the concrete slab of the feed bins and the tire tracks left on the pathways by the animal feed truck, the pig delivery truck and the carcass knacker truck. Thirteen samples were collected per farm per visit for a first subtotal of 390 samples (13 samples per visit × 10 farms × 3 visits).

Thirty additional samples (5 vehicles × 2 samples per visit × 3 visits) were collected by swabbing a 30 cm × 30 cm surface from mudguards and cabin carpets of the veterinarian and technician vehicles on their arrival at the farm.

In addition, on two separate occasions on all 10 farms, right before loading the pigs on the trucks for transportation to the slaughterhouse, samples were collected by swabbing a 30 cm × 30 cm surface of the mudguards and the empty truck box. Here, 40 more samples (10 farms × 2 visits × samples) were collected.

Finally, the efficiency of the pig transporter to eliminate the *Salmonella* contamination on selected locations on the trucks was measured twice, once in winter, once in summer, by swabbing a 30 cm × 30 cm surface from the mudguards, the empty box and the cabin carpet right after the cleaning, disinfection protocol. Here, 24 more samples (four transport trucks × two seasons × three samples) were taken.

In total, 484 samples were collected, chilled in ice, transported to the laboratory and analysed for the presence of *Salmonella*.

Isolation of *Salmonella*

Isolation of *Salmonella* was done according to The International Organization for Standardization ISO 6579:2002+Amd 1:2007.¹⁶ Briefly, each sample was pre-enriched in buffered peptone water and incubated at 37°C overnight (18–20 hours). Next, each culture from the pre-enrichment broth was inoculated in Rappaport-Vassiliadis medium with soya (RSV broth) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn broth, pH 8.0). The RSV and MKTTn broths were incubated at 42°C and 37°C overnight, respectively. Cultures were plated on Xylose lysine deoxycholate agar (XLD agar) and incubated at 37°C overnight. In some cases, colonies on XLD agar were obtained from both RSV and MKTTn broths. In these cases, both colonies were

further characterised. Identity of *Salmonella* isolates was confirmed by biochemical tests including Triple Sugar Iron agar reaction, Lysine Iron Agar and urease^{1 17 18} and API 20E (Biomérieux Canada, Montreal, Quebec, Canada) according to the manufacturer's instructions. All confirmed *Salmonella* isolates were sent to 'Laboratoire d'épidémiologie animale du Québec' (MAPAQ, Saint-Hyacinthe, Quebec, Canada) for serotyping.¹⁹ *S. Typhimurium* isolates were further characterised by lysotyping²⁰ at the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg, Manitoba, Canada). All comparisons of *Salmonella* isolate detection rates were done using Fisher's exact test.²¹

Restriction fragment length polymorphism (RFLP)-Pulsed-field gel electrophoresis (PFGE) genotyping of *Salmonella*

RFLP-PFGE genotyping was carried on selected *S. Derby* strains according to the standardised CDC PulseNet protocol^{22–24} with the restriction enzymes XbaI and BlnI. DNA patterns were visually compared.

RESULTS

Distribution and identification of *Salmonella* on the 10 farms

Ten farms were visited three times over a 10-month period. The 390 samples collected on the farms were analysed for the presence of *Salmonella*. The bacterium was isolated on 9 of the 10 farms and in 39 of the 390 samples. Here, 42 *Salmonella* isolates were obtained (table 1). They encompassed seven different serotypes: Derby (14 isolates), Typhimurium,¹⁰ Infantis,⁸ Branderburg,⁶ Senftenberg,¹ Agona¹ and Mbandaka¹ and one autoagglutinable isolate (a rough variant of *Salmonella* that did not express O antigen). Five different *S. Typhimurium* lysotypes, 12 (2 isolates), 104,¹ 193,⁴ 208,² and the monophasic *S. typhimurium* variant 1,4,[5],12:i-¹ were detected.

Some farms yielded more *Salmonella* isolates than others. At one end of the spectrum, the three visits at farms F and E yielded 12 and 8 isolates, respectively, whereas at the other end (Fisher's exact test; $p < 0.05$), farm G was *Salmonella* free on all three visits for the samples tested.

Differential distribution of *Salmonella* over the three visits

In many cases, presence of *Salmonella* on objects and locations inside and outside the barn on a specific farm, A–J, was restricted to only one of the three visits. On farm A, during the first visit (A1), the feed pipeline yielded a *Salmonella* isolate, whereas *Salmonella* was not detected from this feed pipeline during visits 2 and 3 (A2 and A3). Here, however, *Salmonella* was isolated from the landing stage on farm A during visits 1 and 2 (A1 and A2), but not during visit 3 (A3). Interestingly, the first visit on farm E yielded two *Salmonella* isolates, *S. Typhimurium* lysotype 104 from the landing stage inside the barn and *S. Agona* from the tire tracks left on the pathway of the animal feed truck. However, the second visit on farm E yielded six isolates, four from the faeces, a fifth one from the mobile objects and a sixth one from the tire tracks left on the pathway of the pig delivery truck. The

contamination seen on specific locations on the first visit had disappeared, but new contaminations were detected elsewhere. Here, five of the six isolates were *S. Brandenburg*, the sixth was *S. Derby*. No *Salmonella* was detected on the third visit on farm E. Somehow, *S. Brandenburg* was present on multiple objects and locations on farm E during visit two but was undetected during visits one and three. Interestingly, although all 10 farms were linked to the same cooperative and they all shared several common resources, *S. Brandenburg* was not detected on the other nine farms on each of the three visits, except on farm H on the first visit, and only on the landing stage inside the barn. Likewise, the first visit on farm F yielded two *S. Infantis* isolates – one from mobile objects, the other from the landing stage inside the barn. On the second visit on farm F, *S. Infantis* was again isolated but from faeces, pools 2–5 and again from the landing stage inside the barn. On the third visit, five *Salmonella* isolates were obtained from farm F, *S. Typhimurium* lysotypes 193 and 208 from the landing stage inside the barn, *S. Infantis* from tire tracks left on the pathway of the pig delivery truck and *S. Typhimurium* lysotypes 12 and 193 from tire tracks left on the pathway of the carcass knacker truck. *Salmonella* was not detected in the faeces and on the mobile objects. Interestingly, *S. Infantis* was not isolated from the other nine farms during all three visits possibly ruling out here systemic cross-contamination between farms interconnected within the same cooperative. However, *S. Typhimurium* lysotypes, 12, 193 or 208, were also isolated on the third visit on farms A and C but not on the other farms. Interestingly, *S. Derby* was detected on 6 of the 10 farms studied and on at least two successive visits on farms A, B and D. Although successive contaminations on one farm may question the efficiency in the local mitigation measures, the wider distribution of *S. Derby* isolates on this network of farms with 13 isolates/42 total isolates may suggest cross-contamination or contamination from a common origin within the network for this specific serotype.

Differential *Salmonella* contamination of locations and objects on the farms

Some locations and objects were more contaminated than others; they yielded more *Salmonella* isolates. Inside the barn, *Salmonella* was mostly isolated from the landing stage (14 isolates) (Fisher's exact test; $p < 0.05$) and to a lesser extent from the faeces (five samples on each farm yielded a total of 13 isolates), the mobile objects,⁵ the barn front door handle¹ and the 100 cm section of the feed pipeline.¹ The mobile objects are restricted to their respective farm and different *Salmonella* serotypes and lysotype were isolated indicating different origins. The mobile objects can also reveal the persistence of specific *Salmonella* on some farms, as exemplified by the presence of *S. Derby* on visits 1 and 2 on farm D. Outside the barn, the tire tracks left on the pathways by the carcass knacker truck (four isolates) and the pig delivery truck³ yielded the highest number of *Salmonella* isolates,

followed by the tracks left on the pathways by the animal feed truck.¹ *Salmonella* contamination was not widespread but was rather restricted to some objects and locations, often during only one of the three visits, and on a small number of farms. *Salmonella* isolates covered different serovars, suggesting no or very limited cross-contamination between farms. No *Salmonella* was detected from the concrete slab of the feed bins.

Differential *Salmonella* contamination inside and outside the barn
Salmonella strains were isolated inside the barn (34 isolates) and outside.⁸ The identities of the *Salmonella* serotypes and *S. Typhimurium* lysotypes varied accordingly. Whereas most *S. Brandenburg*, *S. Derby* and *S. Infantis* were isolated inside the barn, *S. Agona*, *S. Mbandaka* and *S. Typhimurium* lysotype 12 were only isolated outside the farms.

Distribution and identification of *Salmonella* from the mudguards and cabin carpets of the veterinarian and technician vehicles

Of the 30 samples tested from truck mudguards and carpets from the driver's cabin of the veterinarian and technician vehicles, a single mudguard sample tested positive and contained *S. Derby*.

Distribution and identification of *Salmonella* from selected locations on the pig transportation trucks

Of the 40 samples tested from the empty box and the mudguards of the transportation trucks, only three box samples were positive for *Salmonella*. They contained *S. Derby*, *S. Agona* and *S. Brandenburg*, respectively. The 20 truck mudguard swabs did not reveal the presence of *Salmonella*.

Distribution and identification of *Salmonella* from selected locations on the pig transportation trucks after the cleaning, disinfection protocol in winter and summer

The transporter's efficiency in eliminating *Salmonella* from its truck with a cleaning, disinfection protocol was assessed. In winter, immediately after cleaning and disinfection, no *Salmonella* was detected in all parts sampled: the truck empty box, the cabin carpet and the mudguard. In summer, however, a positive sample was obtained from a truck box and identified as *S. Brandenburg*, and two more samples were obtained from a second truck, the cabin carpet and the mudguard and identified as *S. Derby* and *S. Typhimurium*, respectively.

RFLP-PFGE genotyping of *Salmonella*

The genetic diversity of a subset of our *S. Derby* isolates was characterised by RFLP-PFGE. Interestingly, all *S. Derby* isolates analysed shared similar XbaI or BnII profiles (figure 1). At least 44 different *S. Derby* PFGE XbaI and BnII profiles are known.²⁵ Here, despite the different sources of our *S. Derby* isolates, different farms, different visits, their similar PFGE profiles for both enzymes strongly suggest a single origin. This origin is yet unknown. The distribution of this specific *S. Derby*

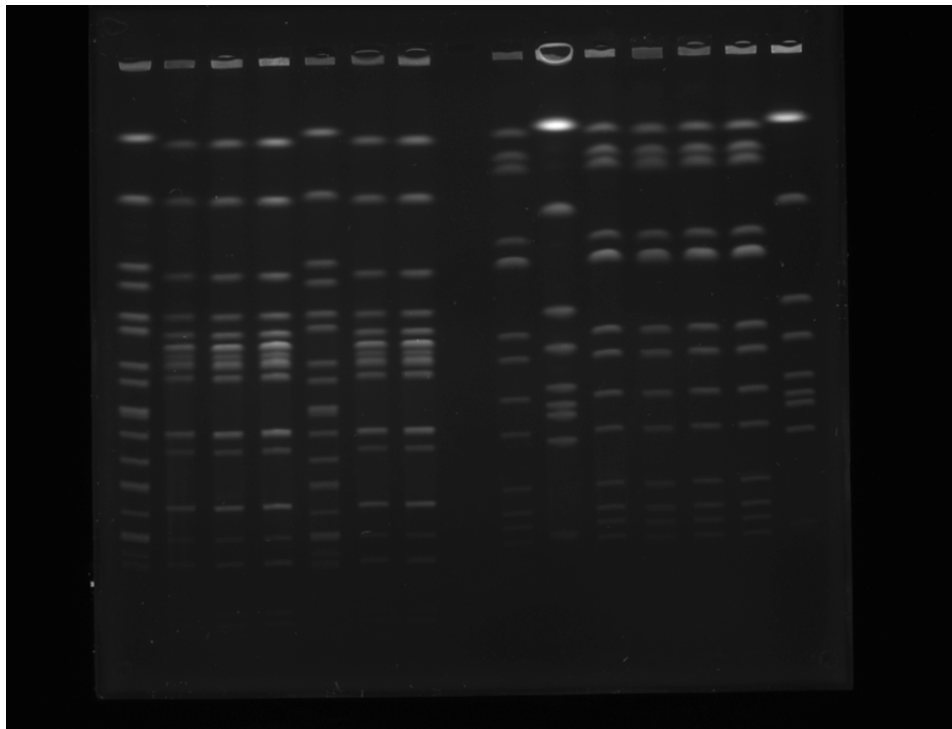


Figure 1 PFGE of *S. Derby* isolates from farm landing stages. Lanes 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 Pulse-Field-Gel-Electrophoresis analysis of *Salmonella* isolates found on three farm landing stages inside the barn. Lanes 1–7: DNA restricted with *Xba*I; lane 1: control, *S. Braenderup*, 2: *S. Derby* from farm a visit 1, 3: *S. Derby* from farm a visit 2, 4: *S. Derby* from farm B visit 1, 5: control, *S. Braenderup*, 6: *S. Derby* from farm D visit 1, 7: *S. Derby* from farm H visit 2, 8: empty; lanes 9–15: DNA restricted with *Bln*I; lane 9: *S. Derby* from farm a visit 1, 10: control, *S. Braenderup*, 11: *S. Derby* from farm a visit 2, 12: *S. Derby* from farm B visit 1, 13: *S. Derby* from farm D visit 1, 14: *S. Derby* from farm H visit 2, and 15: control, *S. Braenderup*.

pulsovar outside this network of 10 pig farms is also unknown.

DISCUSSION

We have shown here that *Salmonella* can occasionally be isolated from a network of farms linked to a same cooperative. *Salmonella* can be isolated inside the barn, on the landing stage, in faeces and on some mobile objects. The landing stage was the location with the highest number of swab samples positive for *Salmonella* across all farms. Regrettably, this location is barely mentioned in other studies on *Salmonella* contamination in swine farms. Yet, landing stages are the first contact of piglets when entering the farm and the fattening barn. Naïve pigs in contact with this contaminated environment could rapidly become *Salmonella* positive and shedders.^{26,27} Interestingly, *S. Derby* was mostly isolated from the landing stages across a number of different farms and visits, with exceptions, and it was always found inside the barn, not outside. We showed that these *S. Derby* isolates are genetically similar and are presumably of clonal origin. Clearly, our study indicates that a swine farm cleaning and disinfection protocol should include a thorough cleaning of the landing stages. Conversely, most other *Salmonella* serotypes and lysotypes isolated in our study were mostly restricted to a single farm. This is interesting given that the 10 farms under study consisted of a network interconnected within the same cooperative. Here, the presence

of specific *Salmonella* restricted to a single farm on a single visit indicates the efficiency of the mitigation measures in cleaning and preventing the persistence of *Salmonella*.

Outside the barn, the tire tracks left on the pathways by the animal feed truck, the pig delivery truck and the carcass knacker truck can also carry *Salmonella* suggesting that the tires of either truck could be contaminated, thus potentially bringing contamination from the outside to the farm, or alternatively, the tires could become contaminated at the farm and carry *Salmonella* elsewhere, perhaps to another farm or to the slaughterhouse, both components of the network here. This is not what we saw, with an absence of common *Salmonella* serotypes or lysotypes on tire tracks on other farms. *Salmonella* dynamics associated to transport appeared limited.

At the farm, mudguards and carpets from the driver's cabin of the veterinarian and technician vehicles did not appear as a source of *Salmonella* contamination. Likewise, of the items tested on the transportation trucks, the mudguards did not appear as a source of contamination, but the empty box may, at times, be one. Here also, a cleaning and disinfection protocol should prioritise a thorough cleaning of the truck box. Several other parts of the transportation truck were not tested for the presence of *Salmonella*: the carpet inside of the cabin among others. Given that these carpets are in contact with boots that have walked several pathways on the pig farm, outside and inside the barn, and elsewhere on the network,



including the slaughterhouse, it is likely they come in contact with *Salmonella* and potentially become carrier. Other parts of the transportation trucks, yet to be characterised, could also be contaminated with *Salmonella*.

In conclusion, our work shows that good biosecurity standards²⁸ were well implemented on the 10 farms studied here, interconnected within a common cooperative, and have proven effective, with the possible exception of the pig landing stage inside the barn on some farms where more attention should be paid.

We are planning to follow-up on this work by studying the presence of *Salmonella* at the pig slaughterhouse from this same cooperative. Unusual objects and locations, yards, trucks, mobile objects and so on will be sampled. *Salmonella* Derby isolates will be characterised by RFLP-PFGE and the profiles compared with the ones revealed here. One of our goals includes the study of the distribution of *Salmonella* in the Canadian swine industry to help control potential disease outbreaks.

Contributors The study was designed by AH, AL and PF. All authors made substantial contributions to the acquisition of data, their analysis and interpretation. The manuscript was drafted by J-CC, AH and PF. All authors revised the manuscript critically and gave final approval of the version to be published.

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Data availability statement All data relevant to the study are included in the article.

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ORCID iD

Philippe Fravallo <http://orcid.org/0000-0003-1796-0852>

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