Investigation of the potential effects of transportation and lairage on the contamination of pig carcasses with Yersinia enterocolitica

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Abstract

Pathogenic Yersinia enterocolitica is frequently isolated from pig tonsils, but can also be found in the feces and lymph nodes of infected animals; which represent potential sources for the surface carcass contamination during slaughter. The aim of the study was to investigate the respective effects of the transportation and lairage steps on the overall contamination of the carcass, taking also into account the contamination of their environment. Each Trial was conducted in two abattoirs, whose environmental contamination was assessed. In each abattoir, 6 trucks and 6 lairage pens were sampled in duplicate at the end of the day, for 5 consecutive days, in spring and autumn. Four groups of 8 pigs from the Anses Specific Pathogen-Free herd (SPF) were mixed with conventional pigs for different contact times during transportation (1h) or lairage (2, 4 or 8h) prior to slaughter, and one group had no contact with other pigs. Each group of SPF pigs was sampled at the end of the slaughter line for the presence of pathogenic Y. enterocolitica for internal (tonsils, caecal content, mesenteric lymph nodes) and external (carcass surface) contamination. Samples were placed in ITC broth for enrichment at 25°C for 48h. Broth was streaked on CIN plates. After 24H at 30°C, typical colonies were streaked on YeCM plates. Biochemical tests were done to confirm Y. enterocolitica and to identify the biotype. The environmental contamination of trucks and lairage was very low. Y. enterocolitica was only detected in 11/240 lairage swabs. All internal and external samples from SPF pigs mixed with conventional pigs during transportation or lairage were negative after slaughter. Four tonsils of the SPF pigs slaughtered directly on the slaughter line after 4 hours of activity, with no prior contact with conventional pigs, and one of the corresponding surface carcasses, were found positive in one abattoir. In our experimental conditions with SPF pigs, we were not able to demonstrate contamination by pathogenic Y. enterocolitica.

Introduction

In 2013, yersiniosis was the third most frequently reported human zoonosis in Europe, with a total of 6 471 confirmed cases (EFSA, 2015). Pigs are frequently described as a source of human contamination by Yersinia enterocolitica through consumption of raw or undercooked meat (Satterthwaite et al., 1999). Pigs do not develop clinical signs, but they carry Y. enterocolitica in their oral cavities, on their tongues and tonsils, and in their lymph nodes, and they excrete this bacterium in their feces (Nesbakken et al., 2003). From a one-survey carried in France on 3120 pigs from 96 batches tested in 16 slaughterhouses (Fondrevez et al., 2014), 13.7% of the pigs were found positive for pathogenic Y. enterocolitica on their tonsils and 74.3% of the pig batches contained at least one positive pig. Presence of Y. enterocolitica on pig tonsils or feces is a potential source of contamination of the carcasses during slaughter. The contamination of Y. enterocolitica free-pigs can also occur during transport (trucks) and during lairage (pens) through the immediate environment of pigs, by contact or ingestion of infected feces from other pigs. The aim of the study was to investigate the respective effects of the transportation and lairage steps on the overall contamination of the carcass, taking also into account the contamination of their environment.
Material and Methods

_Y. enterocolitica_ status of trucks and lairage pens.

The trial was conducted in two abattoirs (AB1 and AB2; more than 600 pigs/h) of West of France, on 2 different weeks (one week in spring and one week in autumn). In each abattoir, a total of 60 trucks were sampled; 6 trucks per day, during 5 consecutive days, on the two weeks. Trucks were taken at random after 6 to 8 hours of activity. After the unloading of the animals and before the truck was washed, each truck was swabbed with two swabs. Each swab was applied on one floor of the truck on 4 different zones of 0.25m². In each abattoir, a total of 60 lairage pens were sampled; 6 lairage pens per day, during 5 consecutive days, on the two weeks. Lairage pens were taken at random after 6 to 8 hours of activity. After departure of a batch of pigs and before arrival of other animals, each lairage pen was swabbed with two swabs. Each swab was applied on the floor on 4 different zones of 0.25m². The floor types of lairage pens were solid concrete for the AB1 abattoir and concrete slatted floor for the AB2 abattoir. In these abattoirs, the pens underwent once a week a full cleaning and disinfection procedure at the end of the week.

Transmission of the _Y. enterocolitica_ during transportation, lairage waiting and slaughter

To evaluate the risk of transmission of the bacteria to _Y. enterocolitica_-free pigs during transportation, lairage waiting and slaughter, Specific Pathogen-Free animals (SPF pigs from Anses) were slaughtered in each of the two abattoirs, AB1 and AB2, according 3 modalities. M1 (contact during transportation): 8 SPF pigs were loaded in last position in a truck containing conventional pigs from another farm and stayed in contact in the truck with these pigs during 2 hours before being directly slaughtered in first position at the beginning of slaughter, without waiting time in lairage. M2 (contact during lairage): 3 groups of 8 SPF pigs arrived respectively at the abattoir 2, 4 and 8 hours (M2-2H, M2-4H, M2-8H) before the beginning of the slaughter. They were transported in a clean and disinfected truck of Anses. They were mixed in one lairage pen with conventional pigs from another herd. There were slaughtered in first position. The floor types of lairage pens were solid concrete for the AB1 abattoir and concrete slatted floor for the AB2 abattoir. M3 (contact during slaughter): 8 SPF pigs arrived at the abattoir after 4 hours of slaughtering activity. They were transported in a clean and disinfected truck of Anses and had no contact with other pigs. They were slaughtered directly without waiting time in lairage. For all the modalities, 20 swabs of the abattoir environment (equipment, machines, tools...) were realized after cleaning and disinfection, or just before the slaughtering of the SPF pigs. Several samples were collected from the SPF pigs; caecal content and mesenteric lymph nodes were collected after evisceration and, carcass swab on one half of carcass and tonsil swab were realized before the cooling step. All the modalities were realized from Tuesday evening till Wednesday morning. The truck of Anses was always confirmed negative for _Y. enterocolitica_ as well as SPF pigs’ feces and tonsils before departure of the SPF pigs from Anses.

Samples analysis

To detect the bacteria and, to characterize and biotype the isolates, we used the same method as described by Fondrevez et al., (2010); this method was used previously during the one-year survey (Fondrevez et al., 2014). In brief, samples were enriched in ITC broth; 100 ml for environmental and carcass swabs, 9 ml for tonsil swabs, 225ml for 25g of caecal content and for 25g of lymph nodes. After 18h at 25°C, ITC was streaked on CIN plate. Plates were incubated for 24h at 30°C. Typical colonies were isolated on YeCM plate and then on PCA. Biochemical tests were done to confirm _Y. enterocolitica_ and identify the biotype.

Results

The environmental contamination of trucks (0/240 swabs) and lairage (11/240 swabs) was very low. No _Y. enterocolitica_ was detected in the 120 trucks. _Y. enterocolitica_ was detected in only 11 swabs of lairage pens, in the sampling done in autumn. They corresponded to 5 pens for the AB1 abattoir and to 3 pens for the AB2 abattoir. _Y. enterocolitica_ was detected mainly from Wednesday to Friday. Isolates were from biotype 1A and 4 in the AB1 abattoir, and from biotype 1A in the AB2 abattoir. All internal and external samples from SPF pigs mixed with conventional pigs during transportation or lairage (M1 and M2) were negative after slaughter. For the contact during slaughter (M3), three environmental swabs of the abattoirs before activity were positive for _Y. enterocolitica_ biotype 1A (2 swabs for AB1, and one for AB2). After 4 hours of activity, all the environmental swabs of the two abattoirs, sampled before the SPF pigs slaughtering, were negative. Among the 16 SPF pigs tested, slaughtered directly with no prior contact with conventional pigs, four tonsils and one carcass of our SPF pigs were found positive for _Y. enterocolitica_ biotype 4 in the abattoir AB2, due to unidentified cross-contaminations on the slaughter line.

Discussion

Despite a reported high contamination of pig tonsils by _Y. enterocolitica_ (13.7% of pigs and 74.3% of the pig batches), for the French pigs (Fondrevez et al., 2014), we observed in this study a very low presence of the bacteria in the environment of the pigs during transportation and lairage. In a parallel sampling to our study, Leblanc-Mariador et al., (2015) isolated Salmonella in 90 to 100% of the same lairage swabs when pigs were present and in 30 to 60% of the truck swabs. This low environmental contamination by _Y. enterocolitica_ could result from a low qualitative and quantitative presence in the feces of infected pigs and/or a limited ability of the bacteria to survive in these conditions. In Belgium, Van Damme et al., (2015) found a prevalence of 25% in pig feces for a prevalence of 55% in pig tonsils, with a median concentration in _Y. enterocolitica_ of 2.80 log CFU/g of pig feces and of 4.14 log CFU/g of pig tonsil. The lower prevalence for French pig tonsil (13.7%) suggests that the prevalence for pig feces could be lower as well, and consequently little participate to the dissemination of the bacteria in the environment of the animals. Indeed, a presence of 9.5% only was observed in French pig’s feces, in a previous study (Feurer et al., 2012). An experimental contamination of the SPF pigs by _Y. enterocolitica_ confirmed that the bacteria can colonize and be excreted by the pigs (Personal communication, E. Eusnault). Quantitative data (in progress) will specify the level of _Y. enterocolitica_ excretion by the pigs. In our limited experimental conditions with SPF pigs, we were not able to demonstrate contamination by contact with conventional pigs during transportation and lairage steps, while these steps were identified as risk for transmission of _Y. enterocolitica_ in the feces of slaughtered pigs and waiting times in lairage; the risk of infection (or fecal excretion) increasing with longer waiting times. The risk of contamination of non-infected pigs during transportation and lairage appears limited, but a contamination during slaughter could be observed despite a small number of animals tested. Indeed, four of our 16 SPF pigs became positive for tonsil during slaughter and one for carcass. This contamination during slaughter, due to cross-contamination from slaughtered infected animals, confirms that the control of the slaughtering process is necessary. In a process study realized in six abattoirs in France, Feurer et al. (2015) showed that a part of the carcass contamination originated from the pig itself and other part from cross-contaminations on the slaughter line. The presence of _Y. enterocolitica_ on pig carcasses was nearly 5 times higher when the tonsils were already half of the positive carcasses at the end of the slaughter line were due to cross-contaminations. Vilar et al. (2015) and Van Damme et al. (2015) demonstrated that presence of _Y. enterocolitica_ in the tonsils and/or in feces, or in the pluck set, was a risk that the carcass becomes contaminated.
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Discussion

Despite a reported high contamination of pig tonsils by Y. enterocolitica (13.7% of pigs and 74.3% of the pig batches), for the French pigs (Fondrez et al., 2014), we observed in this study a very low presence of the bacteria in the environment of the pigs during transportation and lairage. In a parallel sampling to our study, Leblanc-Maridor et al., (2015) isolated Salmonella in 90 to 100% of the same lairage swages when pigs were present and in 30 to 60% of the truck swabs. This low environmental contamination by Y. enterocolitica could result from a low qualitative and quantitative presence in the feces of infected pigs and/or a limited ability of the bacteria to survive in these conditions. In Belgium, Van Damme et al., (2015) found a prevalence of 25% in pig feces for a prevalence of 55% in pig tonsils, with a median concentration in Y. enterocolitica of 2.80 log10 CFU/g of pig feces and of 4.14 log10 CFU/g of pig tonsil. The lower prevalence for French pig tonsil (13.7%) suggests that the prevalence for pig feces could be lower as well, and consequently little participate to the dissemination of the bacteria in the environment of the animals. Indeed, a presence of 9.5% only was observed in French pig's feces, in a previous study (Feurer et al., 2012). An experimental contamination of the SPF pigs by Y. enterocolitica confirmed that the bacteria can colonize and be excreted by the pigs (Personal communication, E. Esnault). Quantitative data (in progress) will specify the level of Y. enterocolitica excretion by the pigs. In our limited experimental conditions with SPF pigs, we were not able to demonstrate contamination by contact with conventional pigs during transportation and lairage steps, while these steps were identified as risk for transmission of Salmonella between animals (Berends et al., 1996). Van Damme et al., (2014) have meanwhile shown a strong correlation between the presence of Y. enterocolitica in the feces of slaughtered pigs and waiting times in lairage; the risk of infection (or fecal excretion) increasing with longer waiting times. The risk of contamination of non-infected pigs during transportation and lairage appears limited, but a contamination during slaughter could be observed despite a small number of animals tested. Indeed, four of our 16 SPF pigs became positive for tonsil during slaughter and one for carcass. This contamination during slaughter, due to cross-contamination from slaughtered infected animals, confirms that the control of the slaughtering process is necessary. In a process study realized in six abattoirs in France, Feurer et al. (2015) showed that a part of the carcass contamination originated from the pig itself and other part from cross-contaminations on the slaughter line. The presence of Y. enterocolitica on pig carcasses was nearly 5 times higher when the tonsils were sampled than when half of the positive carcasses at the end of the slaughter line were due to cross-contaminations. Vilar et al. (2015) and Van Damme et al. (2015) demonstrated that presence of Y. enterocolitica in the tonsils and/or in feces, or in the pluck set, was a risk that the carcass becomes contaminated.
Conclusion

In our experimental conditions with SPF pigs, we were not able to demonstrate contamination by contact with conventional pigs during transportation and lairage steps, whose environmental contamination appeared to be scarce. Limited cross contaminations were observed during the slaughter process, which emphasizes the importance of good hygiene procedures to limit carcass contamination by pathogenic Y. enterocolitica.

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References


Is the Salmonella contamination of swine carcasses at slaughter related to the Salmonella load in caecum?


Abstract

The aim of this study was to examine the relationship between the load of Salmonella spp. in caeca and the carcass contamination in an Italian slaughterhouse. The sampling scheme was designed to be representative of the pigs slaughtered in a day and to estimate a 12% prevalence of pigs highly contaminated by Salmonella spp. (HCP, cecal load ≥3log). Environmental swabs were taken before slaughter. Cecal contents and carcass swabs were collected from the same pig. Salmonella MPN were estimated according to ISO6579-2:2012/A1 and ISO7218:2007/E. The overall Salmonella prevalence were 34.64% and 7.19% for ceca and carcasses respectively, with S. Derby and S. 4,[5],12:i:- being the prevalent serotypes. The HCP prevalence was 11.44%. 7/59 environmental swabs tested positive; when the same serotype was isolated from the environment and from carcasses, the samples were excluded from further analysis. Statistical analysis was performed to investigate the relationship between Salmonella spp. loads in the cecum and contamination of the carcass of the same pig and the prevalence of HCP and the contamination of carcasses on the same day. For this purpose, the days were classified as “high prevalence days” depending on the proportion of ceca resulted positive (≥36%) and as “high load” days depending on the prevalence of HCP (≥10%). A correlation between the contamination of carcasses and the cecal Salmonella loads of the same animal was found (Spearman’s correlation coefficient: 0.2254; p-value=0.0001). No correlation was found between the contamination of carcasses and the categorization of the day of sampling as “high prevalence day“. Conversely, a correlation was found between the contamination of carcasses and the “high load” category of the sampling day (Wilcoxon test, p=0.0011). Notably, not the prevalence of pigs carrying Salmonella spp. but the prevalence of highly contaminated pigs was shown to be related to the contamination of carcasses.

Introduction

Salmonellosis is still one of the most important foodborne diseases in the EU. Even though the layer hen reservoir remains the most important source of human salmonellosis in the EU, in some countries, including Italy, pork is considered the first contributor to the infection (Pires, 2011). The risk of Salmonella infection in man is not only associated to its presence on carcasses, but also to the bacterium load, since high levels of Salmonella can increase the consumer’s exposure. However, most data on the Salmonella prevalence in the pig production chain are only qualitative, and this can impair quantitative risk assessment (EFSA, 2008). At the slaughterhouse, the source of contamination of carcasses can be from the same animal, from other pigs, or from the environment, a process known as cross-contamination. Salmonella can contaminate the environment either in a persistent way, being present as a ‘house flora’ of the slaughter plant, or in a transient way, by cross contamination from animals slaughtered on the same day (Smid, 2014). In this complex scenario, the intestinal content and the faeces of carrier pigs are, directly or indirectly, the predominant source of Salmonella for carcasses (van Hooeck, 2011). Our hypothesis was that there was a positive association between the Salmonella load in the intestinal content and the contamination of carcasses at slaughter. This association was studied on individuals, correlating the amount of Salmonella in caecum and the Salmonella load of the carcass of the same pig. Then, to unveil the role of intestinal load in cross-contamination, the correlation between the prevalence of highly contaminated pigs and the contamination of carcasses on the same working day was investigated.