Identification and distribution of *E. coli* virulence gene profiles in an operating swine production network.

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Abstract: A number of studies have demonstrated a link between the detection of potentially pathogenic *Escherichia coli* strains and economic loss in the swine industry. *E. coli* strains belong to different commensal or pathogenic clonal groups, the latter being characterized by the presence of specific virulence genes. The transmission of such *E. coli* between herds and a slaughterhouse in a production network, in particular to illustrate the dissemination of *E. coli* strains in a zoonotic perspective, has not been well characterized. The presence of certain virulence genes could be used as indicators of contamination between herds and the slaughterhouse. The objective of this study was to examine some transmission modes of such *E. coli* in a well defined swine production network. A defined region containing 10 farms, a slaughterhouse, and a transportation network was selected. Samples (feces, dust, soil….) were collected at various sites on the farms (3 visits), at the slaughterhouse (2 visits), and on the vehicles of stakeholders linking the farms and slaughterhouse, such as animal transporters. Three consecutive production batches were followed during 8 months. The presence in the samples of virulence genes (eltB, estA, estB, faeG, stxA, stx2A, eae, cnf, papC, iucD, and tsh) commonly associated with pathogenic *E. coli* was examined by conventional multiplex PCR. The monitoring of the virulence gene profiles both temporally and spatially resulted in the identification of an ETEC:F4 profile as such a marker. The distribution of ETEC:F4 suggests that the slaughterhouse yard acts as a reservoir of contamination in the network, ETEC:F4 being transmitted back to the farms by mechanical vectors. These results illustrated the need to improve the biosecurity relationship between herds and slaughterhouse, both playing a role in distribution of pathogens in pig production.

Introduction: *E. coli* is known to be an important constituent of the pig intestinal microflora. Most of the isolates colonize the small intestine and are commensal. However, some isolates encode virulence genes thus may be pathogenic and potentially zoonotic. Certain *E. coli* pathotypes cause post-weaning diarrhea, an important cause of mortality in pigs (Fairbrother et al., 2007). The transmission of such *E. coli* between herds and the slaughterhouse in a given network, in particular to illustrate the dissemination of *E. coli* strains in a zoonotic perspective, has not been well characterized. The presence of certain virulence genes could be used as an indicator of contamination between herds and the slaughterhouse. The objective of this study was to examine some transmission modes of such *E. coli* in a well defined swine production network. The monitoring of the virulence gene profiles both temporally and spatially resulted in the identification of an ETEC:F4 contamination profile.

Material and Methods: A swine production network consisting of 10 finishing farms, a transportation network and a slaughterhouse was selected. A total of 388 environmental samples were taken at the farms during 3 visits representing respectively 3 successive production batches. The farm environmental samples consisted of pools of feces from healthy pigs, swabs of objects and tools inside the farm buildings (panels, scales, working desks, high places, loading dock floor) and some surfaces of vehicle tracks outside the farm (animal departure, feed, knackery). Also, 272 environmental samples were taken in the slaughterhouse field following transport of 2 production batches from each of 9 of the 10 farms. The slaughterhouse environmental samples were taken on the departure dock, inside the truck cabin before loading and on the truck mud-guards and vehicle tracks at different times during the departure procedure. Samples were incubated 24 hours in a non-selective pre-enrichment broth at 37°C then transferred, respecting a 1:10 ratio, in an enrichment broth for another 24 hours. The cultured samples were centrifuged at 12000 rpm for 5 min, washed in buffer and placed at 100°C for 10 min to prepare the DNA templates for PCR. The presence in the samples of virulence genes (eltB, estA, estB, faeG, stxA, stx2A, eae, cnf, papC, iucD, and tsh) commonly associated with pathogenic *E. coli* (ETEC, ExPEC, STEC and EPEC) was examined by conventional multiplex PCR according to a protocol of the Reference Laboratory for *Escherichia coli* (EcL – Faculté de Médecine Vétérinaire de L’Université de Montréal) available at http://www.apzec.ca/en/APZEC/Protocols/APZEC_PCR_en.aspx. The distribution of *E. coli* virulence genes present in the samples was monitored and analyzed in a spatial and a temporal perspective.
Results: The distribution of *E. coli* virulence genes in the farm samples differed between the pathotypes and the farms. The ETEC virulence gene distribution on the farms was non-homogenous and showed a different contamination profile for each farm. The virulence genes encoding STb and STa toxins are detected throughout the network. On the other hand, the genes encoding for F4 and LT were only detected on certain farms. The detection of environmental samples positive for at least one ETEC toxin in combination with the gene encoding the fimbriae F4 suggests the presence of ETEC: F4+ strains in these samples. The distribution of samples positive for ETEC and F4 was analysed spatially and temporally to describe events of contamination in the network.

Samples positive for ETEC and F4 were detected in feces, objects and the vehicle tracks in most farms in the network, most commonly on farms A, B and H (Table 1). Few or no samples positive for ETEC and F4 were observed on farms D, E, F, G, I and J (results not shown). The spatial distribution of such samples positive for ETEC and F4 indicates the presence of contamination in the intestinal microflora of the pigs, but also in the farm environment and on vehicle tracks.

Most samples positive for ETEC and F4 were associated with the second farm visit (Table 2). This visit occurred in approximately the same period of time for every farm. These results suggest that most farms in the network were more contaminated by ETEC: F4 strains in that same period. Nevertheless, farm A still showed the highest level of ETEC: F4 contamination.

In the slaughterhouse environment, STEC and EPEC virulence genes were detected in a low proportion of samples, although Stx1 was detected more frequently than on the farms, particularly for departure visits associated with farms I and J. Interestingly, in contrast to farm results, ETEC virulence genes were detected on every departure visit to the slaughterhouse (Table 3).

The spatial distribution analysis of samples positive for ETEC and F4 permitted the identification of possible contamination sources in the network. ETEC and F4 were detected on objects and tracks at every delivery visit, but less frequently (one detection or less) for visits on farms D, E, and I (results not shown). Interestingly, delivery visits for farms A, B, C, G and J demonstrated higher ETEC: F4 contamination levels than other visits (Table 3). Hence, ETEC: F4 strains present in the environment could be transmitted by cross contamination to a vector such as the swine transporter and be brought to other farms and/or slaughterhouses.

<table>
<thead>
<tr>
<th>Table 1. Spatial distribution of samples positive for ETEC and F4 in the farm environment on 3 visits.</th>
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<tbody>
<tr>
<td><strong>Total of samples</strong></td>
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<tr>
<td>Farm A</td>
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<td>Farm B</td>
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<td>Farm C</td>
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<tr>
<td>Farm H</td>
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<td><strong>Total</strong></td>
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<th>Table 2. Temporal distribution of samples positive for ETEC and F4 in the farm environment.</th>
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<tbody>
<tr>
<td><strong>Total of samples</strong></td>
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<tr>
<td>Farm A</td>
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<td>Farm B</td>
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<td>Farm C</td>
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<td>Farm H</td>
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<td><strong>Total</strong></td>
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<th>Table 3. Spatial distribution of samples positive for ETEC and F4 in the slaughterhouse environment on 2 visits.</th>
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<tbody>
<tr>
<td><strong>Total of samples</strong></td>
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<td></td>
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<tr>
<td>Delivery farm A</td>
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<tr>
<td>Delivery farm B</td>
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<td>Delivery farm C</td>
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<tr>
<td>Delivery farm G</td>
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<tr>
<td>Delivery farm H</td>
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<td>Delivery farm J</td>
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<tr>
<td><strong>Total</strong></td>
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</table>
The temporal ETEC: F4 virulence gene distribution permitted the identification of the visits when the contamination by the ETEC: F4 profile was higher with respect to the farms associated with the visits. The analysis of this distribution showed that most samples positive for ETEC and F4 were found on the first delivery associated with farms A, B, G and J (Table 4). This result suggests that these farms were a contamination source in the network at this time.

**Discussion:** ETEC: F4 is responsible for post-weaning diarrhea in pigs and its presence can have a great economic impact on production. Hence, it is considered that ETEC: F4 would be a good candidate to describe the transmission of *E. coli* contamination in a production network. Interestingly, the ETEC: F4 profile was more frequently observed in the environment or on objects less accessible to cleaning and disinfection, such as the top of the feeding conveyor and also in the vehicle tracks. This suggests that ETEC: F4 strains may be resistant to desiccation and persist in the farm environment. Such environments could act as a reservoir, permitting transmission of pathogenic *E. coli* strains to pigs of successive batches and from one establishment to another via contamination vectors such as swine transporters. The Stx1 STEC virulence gene was sporadically detected in the slaughterhouse fields. Interestingly, this gene is usually associated with STEC strains found in cattle (Beutin et al., 1993). As the slaughterhouse in our study is only processing swine, it would be important to investigate possibilities explaining the presence of cattle associated STEC isolates in the slaughterhouse environment. A plausible explanation is that swine transporters carry other animal species in other production networks outside of our study.

ETEC virulence genes were detected on every delivery visit to the slaughterhouse. This suggests that the holding field is frequently contaminated with ETEC strains throughout the year. It also strengthens the idea that ETEC: F4 strains are persistent in the environment since their virulence genes are detected in the yards of the slaughterhouse. Also, the slaughterhouse could act as a reservoir of ETEC: F4 strains, as the yards are known to be frequently visited by transporters. Hence, there is a possibility of ETEC: F4 transmission back to the production network via the slaughterhouse field.

**Conclusions and perspectives:** Our approach gives the opportunity to study the distribution of pathogenic *E. coli* virulence genes in a defined network containing farms and a slaughterhouse over a one year period. The virulence gene spatial distribution permitted us to identify an ETEC: F4 profile contamination marker in the network to describe contamination events in the network. In addition, the virulence gene temporal distribution showed when the contamination marker was more or less present in the network. The study of spatial and temporal distribution of samples positive for ETEC and F4 in the production network helped to better understand the transmission mode of pathogenic *E. coli* strains and to prevent possible contamination events in the network. Some farms presented a higher ETEC: F4 level. The slaughterhouse yards appear to be a potential reservoir of contamination. Characterization and comparison of ETEC: F4 isolates from these farms and the slaughterhouse could attest a direct link of contamination. Thus, the next step in this study is to isolate the contamination marker strains in the positive samples taken in the environment of the network. The genotypic characterization of these strains will then link sources with vectors of contamination and contamination events in the network.

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**References:**

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**Table 4. Temporal distribution of samples positive for ETEC and F4 in the slaughterhouse environment.**

<table>
<thead>
<tr>
<th>Total of samples</th>
<th>No. of samples positive for ETEC and F4 (%)</th>
</tr>
</thead>
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<tr>
<td>Visit 1</td>
<td>Visit 2</td>
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<tr>
<td>Delivery farm A</td>
<td>26</td>
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<tr>
<td>Delivery farm B</td>
<td>27</td>
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<tr>
<td>Delivery farm C</td>
<td>31</td>
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<tr>
<td>Delivery farm G</td>
<td>30</td>
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<tr>
<td>Delivery farm H</td>
<td>29</td>
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<tr>
<td>Delivery farm J</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td>272</td>
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