First description of *Campylobacter lanienae* from feces of organic and conventional pigs in France

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French agency for food, environmental and occupational health & safety
Investigate, evaluate, protect

Context of the detection of *C. lanienae* isolates

In order to evaluate *Campylobacter* occurence, antimicrobial resistance and genotypic diversity, fecal samples of 58 pigs from 31 conventional herds and 56 pigs from 31 organic herds, were collected in a slaughterhouse at evisceration step. The analysis of fecal samples was performed as following :

Fecal samples diluted 1: 10 in BPW

Direct detection of *Campylobacter* on Karmali plate

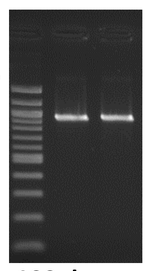
Species identification by PCR Wang (Wang et al., 2002)

Campylobacter coli
76.8 % for organic pigs
74.0% for conventional pigs

BUT 118 isolates with no species identification and classified as « CAH »
(Campylobacter Arcobacter Helicobacter)

Identification with Bruker Maldi Biotyper™
➢ *C. Lanienae* ?

Amplification of 16S rRNA genes
(Logan *et al.*, 2000 et Inglis *et al.*, 2003)
➢ *C. Lanienae*



Sequencing
➢ *C. Lanienae*

Campylobacter occurrence re-estimated :
➢ Organic pigs : 96.3 %
➢ Conventional pigs : 91.1 %

Characterization of *C. lanienae* isolates

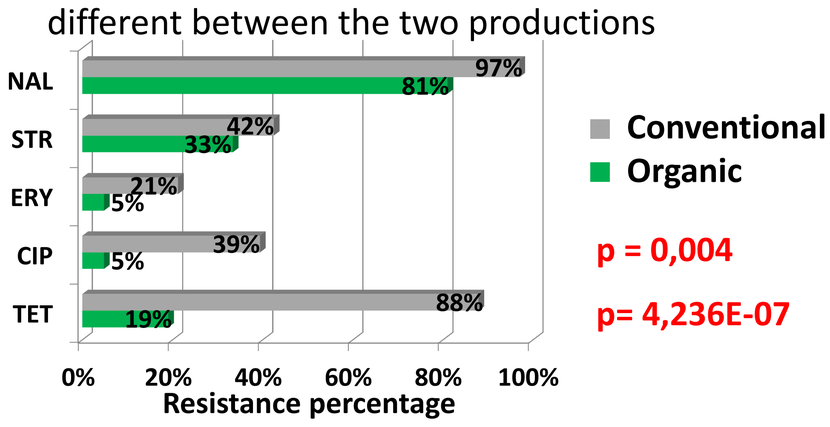
Antimicrobial susceptibility

Method

- 55 *C. Lanienae* studied for their antimicrobial susceptibility by Minimal Inhibitory Concentration (MIC) using Sensititre ® plates (Biocentric, Bandol, France)
- 7 antimicrobials tested : Gentamicin (GEN), Streptomycin (STR), Ciprofloxacin (CIP), Nalidixic Acid (NAL), Tetracycline (TET), Erythromycin (ERY), Chloramphenicol (CHL).
- Results analysed following ECOFFs from Eucast.

Results

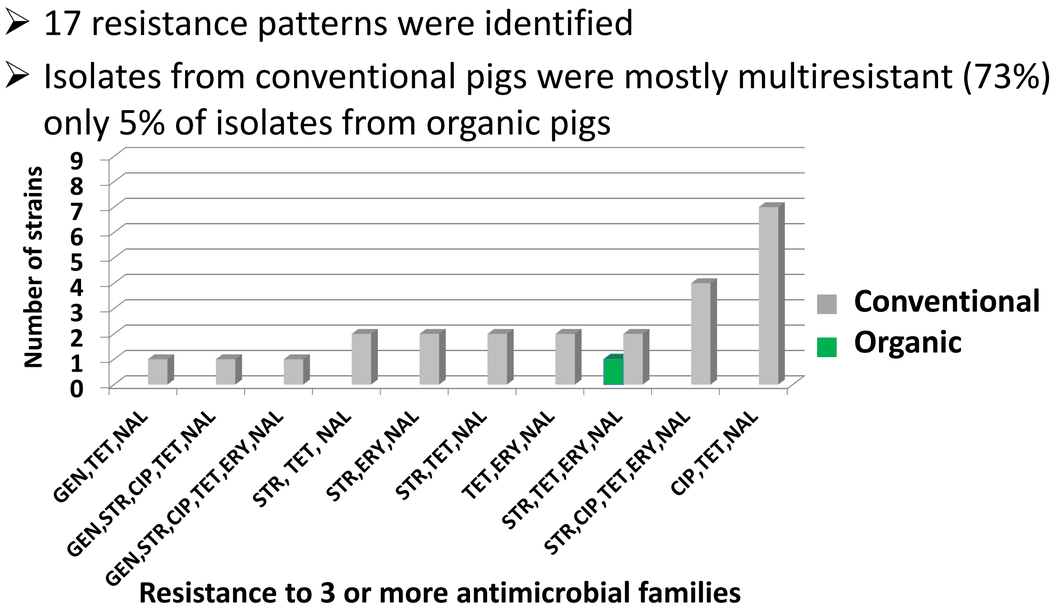
➢ Only one isolate was pansusceptible (1.8%)
➢ All isolates were susceptible to Chloramphenicol and 94.5% susceptible to Gentamycin
➢ Resistance to Nalidixic acid (93 %) is very high : natural resistance
➢ Resistance to Tetracycline and Ciprofloxacine was significantly different between the two productions



p = 0,004

p= 4,236E-07

➢ 17 resistance patterns were identified
➢ Isolates from conventional pigs were mostly multiresistant (73%) *vs* only 5% of isolates from organic pigs

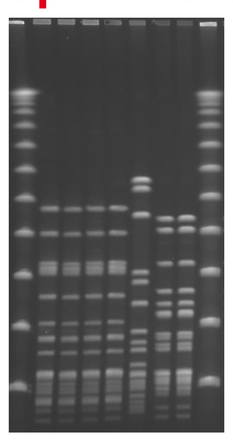


Resistance to 3 or more antimicrobial families

Genotypic diversity : Pulsed-field gel electrophoresis

Method

- DNA preparation, restriction endonuclease digestion and PFGE carried out as described by the Campynet protocol
- DNA macrorestriction performed with *Kpn*I and *Sma*I enzymes.
- Electrophoretic patterns compared using BioNumerics® (Applied Maths, Sint-Martens-Latem, Belgium).
- Simpson's index (D) used to assess the genetic diversity of the Campylobacter populations (Hunter & Gaston, 1988).



Results

➢ High diversity whatever the origin of strains, and the enzyme used (ID > to 0.98)
➢ No interest to use *Sma*I enzyme (lot of strains no typable)

Electrophoretic patterns compared using BioNumerics® software

At 80% of similarity :

➢ 9 clusters

➢ No evidence of genetic clusters linked to a type of production or to a resistance pattern

➢ When isolates showed a same PFGE pattern, they are from the same sample or same herd

➢ The patterns are distinct when they were compared with the others *Campylobacter* species patterns

Conclusion

This study allowed us to demonstrate for the first time in France that pigs, known to be a reservoir for *Campylobacter coli* may also carry in their feces *Campylobacter lanienae*, a species rarely highlighted. The species was present in conventional fecal samples as well as organic fecal samples. The lower level of antibiotic resistance and multiresistance of *C. Lanienae* strains for organic pigs may be related to the restricted use of antibiotics in this production and / or colonization of organic pigs with susceptible environmental strains. The genotypic diversity by RFLP-PFGE is very high, as generally observed for other more common species of *Campylobacter*.

References:
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Logan JM, Burnens A, Linton D, Lawson AJ & Stanley J (2000). *International journal of systematic and evolutionary microbiology* **50 Pt 2**: 865-872.
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Aknowledgement to CORE Organic II Funding Bodies, partners of the FP7 ERA-Net project, for funding SAFEORGANIC project
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