Anses scientific and doctoral days

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Characterization of the *Clostridium perfringens* hazard in the cattle, pig, and poultry sectors in slaughterhouses

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INTRODUCTION

This study is part of the **ANR ClostAbat project** titled "Characterization of the *Clostridium perfringens* and *Clostridioides difficile* hazards in the cattle, pig, and poultry sectors in slaughterhouses".

Clostridium perfringens is a spore forming anaerobic bacteria that is both an ubiquitous environmental bacteria and a foodborne pathogen. 74% of foodborne outbreaks caused by *C. perfringens* in France are associated with the use of contaminated raw materials to prepare mainly meat-based ready meals [1]. *C. perfringens* contamination in the food chain is suspected to occur during the evisceration step in slaughterhouses. However, very few studies have investigated the contamination origins for *C. perfringens*.

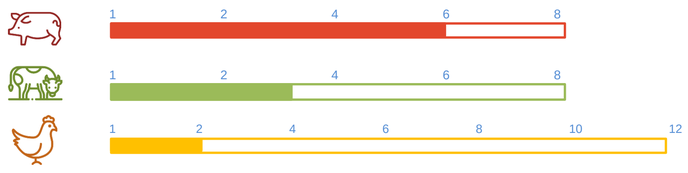
The **objective** of this study is to provide information on prevalence, diversity and pathogenic potential of the *C. perfringens* strains isolated from slaughterhouses.

METHODS

Sampling

Strains will be isolated from 8 to 12 sampling campaigns in 3 sectors: pig, cattle, and poultry. Various types of samples: animal (feces, carcass, meat cuts), environment (work surfaces and operator tools) and air of slaughterhouses and cutting plants.

Current sampling campaigns progress

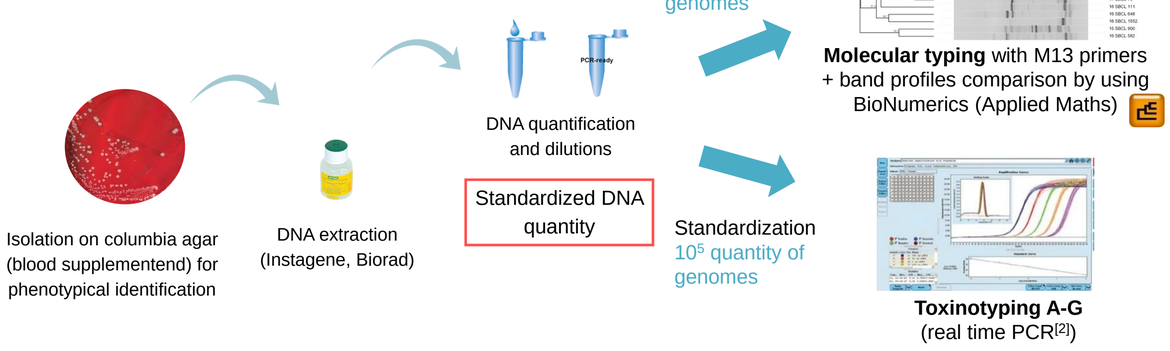


Total isolated strains = 330

Total isolated strains = 42

Total isolated strains = 176

Strains characterization



RESULTS

Prevalence

Prevalence of *C. perfringens* for each sampling campaign in pig, cattle, and poultry sectors

Slaughterhouse for the pig sector	Sampling campaign	Season	Total samples	Positive samples	Prevalence (%)	Slaughterhouse prevalence (%)	Sector prevalence (%)
A	1	Spring	74	9	12	**23 ± 7,5**	**25 ± 10,5**
	3	Fall	74	16	22		
	6	Spring	74	25	34		
B	2	Fall	74	30	41	**25 ± 16**	
	4	Winter	74	7	9		
C	5	Spring	74	23	31	**31**	

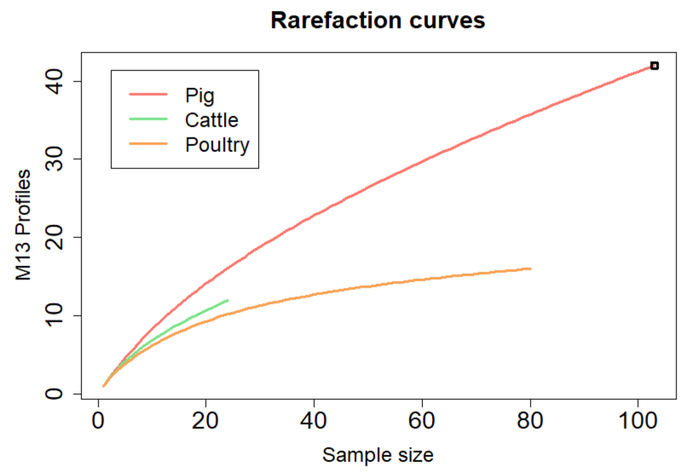
Slaughterhouse for the cattle sector	Sampling campaign	Season	Total samples	Positive samples	Prevalence (%)	Slaughterhouse prevalence (%)	Sector prevalence (%)
D	1	Fall	73	4	**5**	**7 ± 3**	**7 ± 3**
	2	Winter	73	3	**4**		
	3	Winter	73	9	**12**		

Slaughterhouse for the poultry sector	Sampling campaign	Season	Total samples	Positive samples	Prevalence (%)	Slaughterhouse prevalence (%)	Sector prevalence (%)
G	1	Winter	58	17	**29**	**29**	**46 ± 23**
H	2	Winter	58	36	**62**	**62**	

C. perfringens prevalence varies by sector:

- The poultry sector has the highest prevalence of all sectors, with a mean prevalence of 46%.
- The mean prevalence for five sampling campaigns in the pig sector is 25%.
- The cattle sector has the lowest prevalence, with a mean prevalence of 7% for three sampling campaigns.

Diversity



Rarefaction curves for pig, cattle, and poultry sectors

Diversity of *C. perfringens* strains is estimated by comparison of M13 band profiles.

In the pig sector, the diversity of isolates is high (> 40 M13 profiles). The number of distinct profiles increases further with the addition of new strains, suggesting that full diversity has not yet been achieved in this sector.

Conversely, in poultry sector, the diversity of isolates is low (15 M13 profiles). With 80 isolates from two sampling campaigns, the curve begins to flatten at the top, suggesting that full diversity has been achieved for this sector.

In cattle sector, the low number of isolates (25) from the three initial sampling campaigns does not allow to make any conclusion yet.

Toxinotype

Toxinotypes of strains confirmed to be different based on their typing profiles were determined. In pig and cattle sectors, strains are 100% type A. In poultry sector, strains are 76% type A and 24% type G.

Toxinotype of *C. perfringens* strains in pig (n = 125), cattle (n =29), and poultry (n = 63) sectors

	Sector	Toxinotype A (%)	Toxinotype A *cpb2+* (%)	Toxinotype G (%)
	Pig	98	2	0
	Cattle	93	7	0
	Poultry	75	1	24

DISCUSSION & CONCLUSION

C. perfringens prevalence in slaughterhouses in pig, cattle, and poultry sectors are variable and ranges from 4% (cattle slaughterhouse) to 62% (poultry slaughterhouse). Strains diversity are estimated to be high for strains isolated from pig sector, and low for strains from poultry sector. As for toxinotype identification, *C. perfringens* strains are mainly type A. In poultry sector, there are also type G strains (24%). These results, combined with genome sequencing of selected *C. perfringens* isolates, will subsequently enable us to establish whether a relationship exists with strains involved in *C. perfringens*-related human infections.

[1] Santé publique France (SPF). Surveillance des toxi-infections alimentaires collectives (TIAC). Données de la déclaration obligatoire, 2021. 2023.
[2] Abdelrahim, A.M., Radomski, N., Delannoy, S., Djellal, S., Le Négrate, M., Hadjab, et al. 2019. Large-Scale Genomic Analyses and Toxinotyping of *Clostridium perfringens* Implicated in Foodborne Outbreaks in France. Front. Microbiol., 10, 777, doi: 10.3389/fmicb.2019.00777.

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