Implementation of a *Salmonella*-free meat pork production system in Belgium: study plan, methods and preliminary screening results.

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INTRODUCTION

Foodborne toxi-infections caused by *Salmonella* are widespread in industrialized countries. Nothing less than 15 000 cases of human *salmonellosis* are detected each year in Belgium without taking into account patients treated without any diagnostic. According to the Belgian veterinary inspection services, 26.3 % of pig carcasses are contaminated with *Salmonella* out of $11{,}1.10^6$ slaughtered pigs each year. However, *Salmonella* presence in pig intestinal tract doesn't entail necessarily carcass presence and human infection depends on the ingested germ number.

The objectives of this project begun in February 1999 are to obtain and especially maintain a *Salmonella*-free pig production system (4). Besides, implementation and standardization of surveillance methods, microbiological screening and typing should allow immediate reaction.

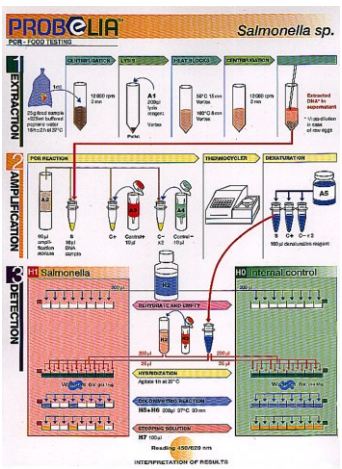
STUDY PLAN AND METHODS

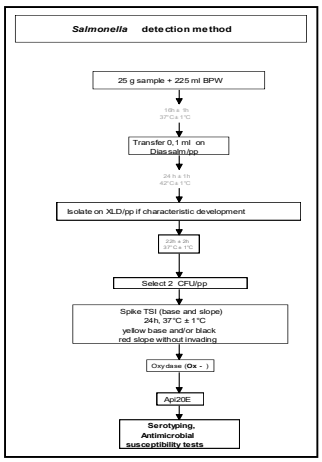
Four major aspects are approached in the study.

The first one consists in the setting up of a questionnaire allowing to put up the situation anamnesis in terms of risk factors in the four different pig production systems. Divided into variable data collected to each visit and non variable data collected once a year, the questionnaire covers sow insemination to fattening pigs including feeding stuffs, carrying, slaughtering, cutting up and commercialization. This suggests precautions and corrective actions to consider for the surveillance plan.

To achieve an efficient surveillance plan, a reliable, sensitive but not time-consuming method is evaluated for *Salmonella* detection. The commercial kit used (Probelia™ *Salmonella sp* amplification kit, Sanofi-Diagnostic-Pasteur, France, figure 1) allow detection in 24 hours. A reference method, using the Diassalm culture media (Diagnostic Semi-Solid *Salmonella* agar, LAB M) (Fig.2), confirms in parallel the PCR results and isolates the strains for further characterizations.

Fig. 1: Probelia™ flowchart. Fig. 2: Reference method flowchart.





Depending on sample types (feed, faeces, waste water (before and after treatment), sludge, pig slurry, meat), optimizations must be carried out to assess inhibition problems and detection limits. Different samples of the same environment are compared to choose the best *Salmonella* recovery method.

Six thousand PCR tests are provided to cover the first 1.5 year surveillance period (distribution in table 1) on a total of 1.10^6 pigs approximately.

Table 1: PCR tests distribution.

Stage	Test number on 78 weeks (%)	Test number / week
Feed	200 (3%)	3
Breeding	600 (10%)	8
Weaning	900 (15%)	12
Fattening	700 (12%)	9
Slaughtering	1980 (33%)	25
Cutting up	160 (3%)	2
Mincing	160 (3%)	2
Commercialization	160 (3%)	2
Waste	300 (5%)	4
Investigations after detection	1000 (16%)	13
Total	6000	78

Nevertheless, the surveillance plan length covers a two-year period preventing seasoning variations. Samples collected every week or once every two weeks will follow piglet's life up to slaughter. Pools of five samples of the same origin are used to increase detection power and decrease costs.

Epidemiological investigations of positive samples will be achieved to trace back the contamination origin thanks to fast molecular biology methods (3) (RAPD (1,2), REP-PCR, plasmid profile,…). Rapidity is of importance for intervention in the field.

Finally, all the information collected from the entire study will be transmitted to the concerned people by the way of guides and reports.

RESULTS

At the present time, the commercial kit gives rise to inhibition problems with pig slurry samples; feed sample analysis lacks of reproducibility with PCR technique. On the other hand, the reference method always provides reproducible results with a detection limit comprised between 1 and 10 CFU/25g of feed and pig slurry samples.

DISCUSSION

Optimizations are necessary to get reproducible and repeatable results before using these methods in surveillance.

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