EXPERIMENTAL STUDY ON THE EVOLUTION OF PIG SLURRY CONTAMINATION BY *SALMONELLA* ENTERICA AND ENVIRONMENTAL IMPACT

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Introduction

Excretion of *Salmonella enterica* by late-fattening pigs may cause the dissemination of this zoonotic agent in the environment during spreading of contaminated slurry (1). Slurry spreading on pastures represents a risk of contamination of animals (ruminants), but also pollution through contaminated runoff water and even pollution of leisure areas (swimming areas) (2). In 2001, an epidemiological study (3) on pig contamination by salmonella led in French breeding-fattening farms showed that 36% of studied farms excreted *Salmonella* in the late fattening period. Even though studies have shown the purifying role of slurry (4), persistence of *Salmonella* in the soil after slurry spreading has been demonstrated (1). Controlling the risk related to the spreading of this effluent entails studying the evolution of *Salmonella enterica* slurry population according to storage time.

A preliminary study was carried out to follow the survival of various serotypes of this zoonotic agent in pig slurry after artificial contamination. Given the results of this study, experimental designs were developed in order to study the effect of different parameters (solid fraction, slurry storage temperature and time, initial *Salmonella* concentration) on the survival of *Salmonella enterica* in pig slurry.

The objective of these studies is to describe the quantitative evolution of different salmonella serotypes, compare two counting methods, describe the influence and interaction of parameters on the survival of slurry *Salmonella*, thereby gathering data that can be applied in the field with a view to propose preventive measures.

Materials and methods

Two series of pig slurry controlled free of *Salmonella* were placed into four flasks amended with four rifampicin-resistant *Salmonella* strains (*Salmonella* Typhimurium, *Salmonella* Brandenburg, *Salmonella* Derby, *Salmonella* Infantis). Using rifampicin-resistant strains makes it possible to compare the mini-MSRV MPN technique (5) to direct isolations on brilliant green supplemented with rifampicin.

Three experimental designs were developed using Doelhert uniform shell design. Three parameters per experimental design were studied at different levels. The number of experiences to be performed was determined according to the following formula: $N=k^2+k+1+n$ (k = number of parameters studied; n = number of replications at the center of the model); i.e., 16 experiments per strain and per experimental design. Two rifampicin-resistant *Salmonella* strains (*Salmonella* Typhimurium and *Salmonella* Brandenburg) were used.

Parameters studied in the first experimental design were initial bacterial concentration (3 levels), percentage of solid fraction (5 levels) and storage time (7 levels from 0 to 30 days). Flasks were amended with *Salmonella* Typhimurium and stored at 20°C. The other two experimental designs studied initial bacterial concentration (3 levels), storage temperature (5 levels) and storage time (7 levels from 0 to 18 days) for two *Salmonella* strains. Flasks were filled with 200 mL slurry controlled free of *Salmonella*, amended with *Salmonella* and stored under the conditions set by the experimental designs. In order to compare the two methods, *Salmonella* counting was done by direct isolation on brilliant green with rifampicin and by mini-MSRV MPN technique.

Results

A 2-log decrease in *Salmonella* population was observed in 28 days for the first series and 35 days for the second. One strain, *Salmonella* Brandenburg, showed a more rapid decrease in both slurries. Comparing count means in paired series ($p<0.05$) brought evidence that mini-MSRV MPN technique is adapted to count salmonella in pig slurry. Moreover, mini-MSRV MPN technique counting is possible with concentrations lower than the limit of numeration by direct isolation. Effluent volume and slurry storage mode (static or under agitation) do not influence *Salmonella* decrease under our experimental conditions. The only difference observed between the two slurries accounting for the difference in behavior was the percentage of solid fraction. This parameter will be included into experimental designs. Slurry *Salmonella* survival also seems to be strain-dependent.

After counting by mini-MSRV MPN technique and processing results using STATGRAPHICS® software, we get the significance (P value) that is set at the limit of 5% and the effects assessed for each parameter, as well as the effect of their interactions on the decrease in the amount of slurry *Salmonella*. In the first experimental design (Table 1), time clearly shows a significant influence on the decrease in *Salmonella* Typhimurium with a negative effect. Solid fraction proportion and initial bacterial concentration do not significantly influence the decrease in *Salmonella* concentration. Only the interaction between time and bacterial concentration is retained with a negative effect.

In the other experimental designs, two strains are compared, *Salmonella* Brandenburg and *Salmonella* Typhimurium (Table 2). Time, which is significant for both strains, has a negative effect. Temperature also has a significant negative effect, but this is only noted for *Salmonella* Typhimurium. For both serotypes, initial concentration does not influence *Salmonella* decrease. Temperature/concentration and time/concentration interactions are significant for *Salmonella* Typhimurium only, with a negative effect.

Table 1: results of experimental design 1

Design 1 *S.* T.	Parameters	P value
Independent parameters	A: Solid fraction	0.8636
	B: Time	**0.001**
	C: Concentration	0.1728
Interactions between parameters	AB	0.9672
	AC	0.9943
	BC	**0.0060**

Table 2: results of experimental design 2

Design 2	Parameters	P value *S.* T.	P value *S.* B.
Independent parameters	A: Temperature	**0.0033**	0.0956
	B: Time	**0.0009**	**0.0026**
	C: Concentration	0.0704	0.3203
Interactions between parameters	AB	0.3293	0.9935
	AC	**0.0155**	0.6469
	BC	**0.0203**	0.4493

*S.*T. *: Salmonella* Typhimurium
*S.*B.: *Salmonella* Brandenburg
Significant effect if P<0.05

Discussion

In all experimental designs and for both strains, time is a significant factor of *Salmonella* concentration decrease.

Temperature is significant with a negative effect for *Salmonella* Typhimurium only. This influence is confirmed by Placha's experiment (4), which describes a more rapid decrease in the summer than in the winter for this bacterium. However, this parameter does not seem to be significant for *Salmonella* Brandenburg; its influence could be strain-dependent.

Initial slurry concentration does not seem to be linked to the phenomenon of bacterial decrease. However, this parameter is still to be measured in order to determine the concentration decrease to be achieved. This observation can be partly explained by the wide limit defined for the time factor. Indeed, the response studied is *Salmonella* concentration in slurry at the time of sampling. The software analyses this response taking into account the three initial parameters, including inoculum. Yet, most results show a very low *Salmonella* count and even a complete absence of *Salmonella* in the analyzed sample, whatever the *Salmonella* amount in the inoculum.

Solid fraction proportion in slurry is a parameter that does not significantly influence the decrease in *Salmonella* concentration. Finally, the effect of time/concentration and temperature/concentration interactions is only significant for *Salmonella* Typhimurium. It may be assumed that there is a strain effect on time and temperature parameters.

Therefore, it would be appropriate to confront some of these parameters, and to include others, in experimental designs carried out over a shorter period so as to validate the effects observed and, subsequently, with different strains in order to observe a possible strain effect linked to the parameters.

Conclusion

Mini-MSRV MPN technique is a pig slurry *Salmonella* counting tool that appears to be reliable, easy to operate, inexpensive and adapted to the field. This technique enables the initial *Salmonella* concentration to be determined in slurry.

Only time stood out as a significant parameter for both strains. The effect of temperature is only noted for *Salmonella* Typhimurium. Beside a possible serotype or strain effect to be confirmed, this difference could also depend on other parameters, such as slurry oxygen content. Thus, new experimental designs will make it possible to confirm these data and quantify the effect of other parameters on the survival of salmonella using field strains as well.

In fact, a final objective is an attempt to develop a mathematical model based on these experimental designs with a view to propose to pig breeders a tool for controlling the risk of dissemination. This mathematical model could contribute to a better control of *Salmonella* dissemination in the environment.

Acknowledgements

ADEME, AFSSE, Porcherie verte, unités HQPAP and EPAQ at AFSSA Ploufragan.

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