Quantification of the effect of factors involved in challenge-test assays on the growth rate estimation of *Listeria monocytogenes*

V. Stahl[1], H. Bergis[2], G. Bourdin[3], M. Cornu[2], C. Denis[4], B. Hezard[1], V. Huchet[5], A.M. Jandos[6], A. Lintz[1], V. Zuliani[7], J.-C. Augustin[8]

[(1)]Aérial, Parc d'Innovation – rue Laurent Fries F-67412 Illkirch (v.stahl@aerial-crt.com)
[(2)] Afssa LERQAP, 23 avenue du Général de Gaulle – F - 94706 Maisons Alfort Cedex (m.simon-cornu@afssa.fr)
[(3)] Afssa LERPPE, Boulevard Bassin Napoléon – F-62200 Boulogne Sur Mer (g.bourdin@afssa.fr)
[(4)]ADRIA NORMANDIE, bd 13 juin 1944 - F - 14310 Villers-Bocage (cdenis@adrianie.org)
[(5)] ADRIA Développement Z.A Creac'h Gwen - F - 29196 Quimper Cédex (veronique.huchet@adria.tm.fr)
[(6)] Institut Pasteur de Lille, 1 rue du Prof. Calmette - BP 245 F - 59019 Lille (anne-marie.jandos@pasteur-lille.fr)
[(7)]IFIP-Institut du porc, pôle viandes fraîches et produits transformés, 7 avenue du Général de Gaulle -F-94704 Maisons Alfort Cedex (veronique.zuliani@ifip.asso.fr)
[(8)] Unité MASQ, Ecole Nationale Vétérinaire d'Alfort, 7 Avenue du Général de Gaulle – F-94704 Maisons-Alfort Cedex (jcaugustin@vet-alfort.fr)

Abstract

This study describes an original interlaboratory trial relative to food challenge-tests conducted by eight French laboratories. The impact of several factors (linked to the type of foodstuff, to biological parameters and to experimental conditions as well as to the laboratory performing the test) on the growth rate of *Listeria monocytogenes* was quantified. The major factors influencing the growth rate variability were the "manufacturing origin of the product" and the "localization of the inoculum in food". Others factors had effects from moderate to high according to the heterogeneity of the food matrix. The factor "laboratory which managed the challenge-test" was not statistically significant. Good laboratories practices, expertise/knowledge of food challenge-tests methodology permitted to control the laboratory effect on the growth rate estimation of *L. monocytogenes.*

Keywords: challenge-test, *Listeria monocytogenes*, growth rate variability, interlaboratory trial

Introduction

Direct evaluation of growth of artificially inoculated bacterial pathogens in foods using challenge-tests is an interesting tool for management of food safety. However, an issue is their ability to describe the growth of a food pathogen in conditions similar as possible to a naturally contaminated and routinely produced food. The laboratory performing a challenge-test must define, according to its expertise and knowledge, different experimental choices. Bacterial growth in food is known to be affected by many factors (Koutsoumanis *et al*., 2004; Tienungoon *et al*., 2000; Devlieghere *et al*., 2001). Models developed in predictive microbiology quantify the effect of temperature, pH, water activity or lactic acid concentration (Zuliani *et al*., 2007; Cornu *et al*., 2006; Le Marc *et al*., 2004). Other factors like competition with simultaneous growth of the food flora are modelled by the use of a new approach (Delignette *et al*., 2006). Sources of variability such as food composition and experimental conditions may have a strong impact on the result of the growth rate estimation of *L. monocytogenes*. In this collaborative research, the objective was to quantify the impact of several factors linked to the type of foodstuff, to biological parameters and to experimental conditions as well as to the laboratory performing the test.

Materials and methods

Experimental design

Seven factors were studied:
Laboratories performing the challenge tests (**factor 1**). Eight French laboratories regularly performing challenge-tests.

Food matrices**.** Between-manufacturers variability **(factor 2)** and between-batches variability **(factor 3).**

Experimental conditions.

*Inoculation method of elaborated products (**factor 4**): in mixed product, on the ingredient the most sensitive to bacterial growth or at interface between ingredients.

*Food structure (**factor 5**): inoculation of the minced or not minced food matrix.

*Food portion sample for *L. monocytogenes* enumeration **(factor 6):** 10g to 270g

*Physiological state of the inoculum **(factor 7):** exponentially growing cells or cells with a nutritional stress (Guillier *et al*. 2006 ; Pinon *et al*. 2004).

Challenge- tests and determination of growth parameters

Experimental plans were designed in relation with specific descriptions of challenge-testing methodologies and were applied to five different foodstuffs: pâté, smoked herring, sliced cooked ham, cooked chicken included in sandwiches and surimi salad. The size of the *L. monocytogenes* inoculum was 100 CFU/g. Challenge-tests parameters, environmental conditions (pH, a_w, temperature 8°C), microbiological flora (total flora, lactic acid bacteria) and artificially inoculated *L. monocytogenes* were monitored along the incubation (shelf-life of the product). Each laboratory estimated the growth rate (μ_{max}) of the obtained growth curves using a fitting tool, Sym'Previus software (www.symprevius.org) which was used by the majority (Pinon *et al.*, 2004).

Statistical analysis

An ANOVA was performed in order to determine which factors had a significant effect on the estimated growth rate.

Results and discussion

1-Laboratory impact

The laboratory which managed the challenge-test had a moderate effect: standard deviations (SD) of μ_{max} range from 0 to 0.010 h^{-1} (Table 1).

Table 1. Effect of the laboratory performing the challenge-test

Food	Labo-ratory	Manu-facturer	Batch	Inoculum location	Portion sample	μ_{max} (h^{-1})	SD
Pâté	1	1	1	whole slice	10 g	0.042	0.003
	2	1	1	whole slice	10 g	0.038	
	1	1	1	stuffing	10 g	0.050	0.002
	3	1	1	stuffing	10 g	0.047	
	2	1	1	interface stuffing/jelly	10 g	0.048	0.005
	3	1	1	interface stuffing/jelly	10 g	0.055	
Smoked herring	1	1	2	surface	10 g	0.021	0.001
	2	1	2	surface	50 g	0.023	
Cooked ham	1	1	1	surface	90 g	0.015	
	2	1	1	surface	270 g	0.029	0.007
	3	1	1	surface	90 g	0.022	
	1	2	1	surface	90 g	0.045	
	2	2	1	surface	90 g	0.030	0.010
	3	2	1	surface	90 g	0.048	
	1	3	1	surface	90 g	0.000	
	2	3	1	surface	90 g	0.000	0.000
	3	3	1	surface	90 g	0.000	
Cooked chicken	1	1	1	surface	25 g	0.058	
	2	1	1	surface	25 g	0.049	0.004
	3	1	1	surface	25 g	0.053	
Surimi salad	1	1	1	surface	25 g	0.071	
	2	1	1	surface	25 g	0.060	0.009
	3	1	1	surface	25 g	0.055	

The Figure 1 shows growth curves obtained under repeatability conditions by different laboratories for the same foods. The average repeatability standard deviation of μ_{max} was on average equal to 0.007 h^{-1}.

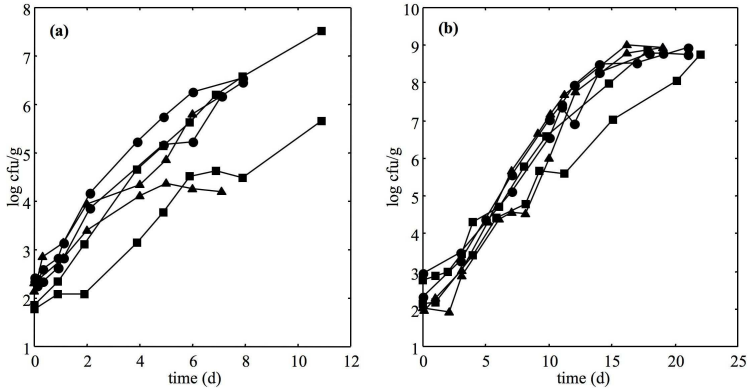

Figure 1. Observed data growth of *L. monocytogenes* at 8°C in (a) surimi salad (b) pâté, from three laboratories (●, ▲, ■) (two repetitions for each laboratory).

2- Food matrix impact

The manufacturer factor had a high impact, dependent on the food studied (SD of 0.001 for smoked herring, 0.021 for sliced cooked ham).

Table 2. Effect of the manufacturer factor

Food	Laboratory	Manufacturer	Batch	Location	Portion sample	μ_{max} (h^{-1})	SD
Smoked herring	1	1	1	surface	10 g	0.027	0.004
	1	1	2	surface	10 g	0.021	
	1	2	1	surface	10 g	0.024	0.002
	1	2	2	surface	10 g	0.021	
	1	1	1 and 2	surface	10 g	0.024	0.001
	1	2	1 and 2	surface	10 g	0.022	
Cooked ham	1	1	1	surface	90 g	0.015	
	1	1	2	surface	90 g	0.023	0.004
	1	1	3	surface	90 g	0.021	
	1, 2 and 3	1	1	surface	90 g	0.022	
	1, 2 and 3	2	1	surface	90 g	0.041	0.021
	1, 2 and 3	3	1	surface	90 g	0.000	
Cooked chicken	1	1	1	surface	25 g	0.053	0.006
	1	1	2	surface	25 g	0.045	
Surimi salad	1	1	1	surface	25 g	0.057	
	1	1	2	surface	25 g	0.045	0.013
	1	1	3	surface	25 g	0.071	

The variability linked to the batches was moderate. The SD was 0.006 on average with SD ranged from 0.002 for the smoked herring to 0.013 for the surimi salad.

3- Experimental conditions impact

The impact of the inoculation method of elaborated products, chosen by the laboratory, led to a SD values ranged from 0.006 to 0.036 h^{-1}. This effect was low for pâté: SD value of 0.006 for the three inoculated zones tested (slice, stuffing, jelly interface). The effect was high for sandwiches: indeed cooked chicken and yoghurt have significantly different physico-chemical characteristics (SD value of 0.036 between the two ingredients with growth on cooked chicken and no growth observed in the yogurt-based sandwhich sauce). The respective effects

of food structure (SD of 0.005 h^{-1} in pâté and smoked herring**)** and of food portion sample for *L. monocytogenes* enumeration were low (SD of 0.004 on average with a SD value of 0.002 for pâté (slice, stuffing) and a SD value of 0.005 for smoked herring).

Conclusion

This original interlaboratory trial relative to food challenge-tests, conducted by eight French laboratories, demonstrated that good laboratories practices, expertise/knowledge of food challenge-tests methodology permitted to control the **laboratory effect** on the growth rate estimation of *L. monocytogenes.* To assess the growth behaviour of *L. monocytogenes* in food, the between manufacturers variability must be considered and their choice explained. The between batches variability was moderate in regard to the objective of a routine challenge test. The impact of the experimental choices like inoculation method was potentially important, and dependent of the type and the heterogeneity of the food matrix. The impact of the food portion sample for enumeration of *L. monocytogenes* was low for the relatively homogeneous products we tested. It may be different for heterogeneous food products.The obtained results will also contribute to the enrichment of French Standard AFNOR NF V01-009 (2007) and the European Technical Guidance Document (Anonymous, 2008).

Acknowledgements

This work was supported by a grant from ACTIA (Paris, France) and the French Ministry of Agriculture, in collaboration with professionals. This project is part of the National French Technological Network (RMT) “Expertise on determination of microbial food products shelf-life”.

References

AFNOR NF V01-009 (2007) describing the laboratory protocols for implementing challenge-tests.

Anonymous (2008) Technical Guidance Document on shelf life studies for *Listeria monocytogenes* in ready-to-eat foods. (http://ec.europa.eu/food/food/biosafety/salmonella/docs/shelflife listeria monocytogenes en.pdf)

Cornu M., Beaufort A., Rudelle S., Laloux L., Bergis H., Miconnet N., Serot T., and Delignette-Muller M.L. (2006) Effect of temperature, WPS (water-phase salt) and phenolic contents on *Listeria monocytogenes* growth rates on cold-smoked salmon and evaluation of secondary models. *International Journal of Food Microbiology* 106, 161– 170.

Delignette-Muller M.L., Cornu M., Pouillot R., and Denis J.B. (2006) Use of Bayesian modelling in risk assessment: Application to growth of *Listeria monocytogenes* and food flora in cold-smoked salmon. *International Journal of Food Microbiology* 106, 195 – 208.

Devlieghere F., Geeraerd A.H., Versyck K.J., Vanderwaetere B., Van Impe J., and Debevere J. (2001) Growth of *Listeria monocytogenes* in modified atmosphere packed cooked meat products: a predictive model. *Food Microbiology* 18, 53-66.

Guillier L. and Augustin J.C. (2006) Modelling the individual cell lag time distributions of *Listeria monocytogenes* as a function of the physiological state and the growth conditions. *International Journal of Food Microbiology* 111 (3), 241-251

Koutsoumanis K. P., Kendall P.A., and Sofos J.N. (2004) A comparative study on growth limits of *Listeria monocytogenes* as affected by temperature, pH and aw when grown in suspension or on a solid surface. *Food Microbiology* 21, 415-422

Le Marc Y., Huchet V., Bourgeois C.M., Guyonnet J.P., Mafart P., and Thuault D. (2002) Combined effects of pH and organic acids on the growth rate of *Listeria innocua*. *International Journal of Food Microbiology* 73 (2-3), 219-237.

Pinon A., Zwietering M., Membré J-M., Leporq B., Mettler E., Perrier L., Thuault D., Coroller L., Stahl V. and Vialette M. (2004) Development and validation of experimental protocols for use of cardinal growth models for prediction of microorganism in food products. *Applied and Environmental Microbiology* 70, 1081-1087.

Tienungoon S., Ratkowsky DA., Mcmeekin TA., and Ross T. (2000) Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. *Applied and Environmental Microbiology* 66**,** 4979-4987.

Zuliani V., Lebert I., Augustin J.C., Garry P., Vendeuvre J.L. and Lebert A. (2007) Modelling the behaviour of *Listeria monocytogenes* in ground pork as a function of pH, water activity, nature and concentration of organic acid salts. *Journal of Applied Microbiology* 103, (2007) 536–550.