

Le directeur général

Maisons-Alfort, le 22 septembre 2017

AVIS

de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail

**suite à la consultation publique de l'Autorité européenne de sécurité des aliments (EFSA)
sur son projet d'avis scientifique « *Listeria monocytogenes* contamination of ready-to-eat
foods and related risks for human health in the European Union »**

L'Anses met en œuvre une expertise scientifique indépendante et pluraliste.

L'Anses contribue principalement à assurer la sécurité sanitaire dans les domaines de l'environnement, du travail et de l'alimentation et à évaluer les risques sanitaires qu'ils peuvent comporter.

Elle contribue également à assurer d'une part la protection de la santé et du bien-être des animaux et de la santé des végétaux et d'autre part à l'évaluation des propriétés nutritionnelles des aliments.

Elle fournit aux autorités compétentes toutes les informations sur ces risques ainsi que l'expertise et l'appui scientifique technique nécessaires à l'élaboration des dispositions législatives et réglementaires et à la mise en œuvre des mesures de gestion du risque (article L.1313-1 du code de la santé publique).

Ses avis sont publiés sur son site internet.

L'Anses s'est saisie le 1^{er} septembre 2017 afin de réaliser une expertise et transmettre les commentaires du comité d'experts spécialisé « Evaluation des risques biologiques liés aux aliments » (CES BIORISK) dans le cadre de la consultation publique organisée par l'EFSA sur son projet avis scientifique relatif à la contamination par *Listeria monocytogenes* des aliments prêts à être consommés et aux risques associés à la présence de ce pathogène pour la santé humaine au sein de l'Union Européenne (UE).

1. CONTEXTE ET OBJET DE LA SAISINE

L'autorité européenne EFSA offre la possibilité, au travers de ces consultations publiques, de partager de manière transparente et de contribuer à des documents scientifiques présentant un intérêt public significatif. Le projet d'avis scientifique du Panel BIOHAZ « *Listeria monocytogenes* contamination of ready-to-eat foods and related risks for human health in the European Union » a été mis en consultation publique le 24 juillet 2017 (transmission des commentaires demandée pour le 29 septembre 2017).

Ce projet d'avis scientifique de l'EFSA fait la synthèse critique des informations européennes disponibles sur *Listeria monocytogenes* dans les aliments prêts à être consommés. Il propose également une analyse des séries temporelles d'incidence de listériose invasive dans l'UE ainsi qu'une évaluation des risques pour la santé humaine associés à la présence de ce pathogène dans les produits prêts à être consommés au sein de l'union européenne.

Le comité d'experts spécialisé (CES) « Evaluation des risques biologiques liés aux aliments » (BIORISK) de l'Anses transmet dans ce document une conclusion synthétique en français des commentaires issus de l'expertise collective. Le détail des commentaires en anglais est disponible en annexe 2, tels qu'ils seront transmis à l'EFSA par voie électronique.

2. ORGANISATION DE L'EXPERTISE

L'expertise a été réalisée dans le respect de la norme NF X 50-110 « Qualité en expertise – Prescriptions générales de compétence pour une expertise (Mai 2003) ».

L'expertise collective a été réalisée par le comité d'experts spécialisé (CES) « Evaluation des risques biologiques liés aux aliments » (BIORISK) sur la base d'un rapport initial rédigé par quatre rapporteurs. Les travaux ont été discutés et adoptés en réunion plénière du 19 septembre 2017.

Toutes les parties du projet d'avis scientifique (parties principales et annexes) de l'EFSA ont été relues et commentées.

L'Anses analyse les liens d'intérêts déclarés par les agents de l'Anses et par les experts avant leur nomination et tout au long des travaux, afin d'éviter les risques de conflits d'intérêts au regard des points traités dans le cadre de l'expertise. Dans ce contexte, un agent de l'unité d'Evaluation des risques liés aux aliments (UERALIM), qui est l'unité en charge de cette thématique au sein de la Direction de l'Evaluation des risques, n'a pas pris part aux travaux et aux délibérations sur cette saisine, du fait de la participation de cet agent aux travaux de l'EFSA. Les déclarations d'intérêts des agents de l'Anses et des experts sont publiées sur le site internet de l'Anses (www.anses.fr).

3. ANALYSE ET CONCLUSIONS DU CES BIORISK

3.1. Synthèse des commentaires sur le projet d'avis scientifique

Les commentaires et remarques sont présentés en anglais dans leur intégralité en annexe 2 de ce document, et suivent le découpage des différentes parties du projet d'avis scientifique du Groupe scientifique sur les dangers biologiques (BIOHAZ) de l'EFSA.

Ces travaux européens confirment l'augmentation de cas de listériose humaine constatée en Europe depuis 2009 (ECDC 2016) et rendent compte de l'évolution de la maladie au sein de différents groupes d'âges entre 2008 et 2015. Dans le cadre de ces travaux, une revue de la bibliographie a été menée et un modèle d'appréciation quantitative du risque (AQR) a été développé afin d'évaluer la contribution des facteurs identifiés comme susceptibles d'expliquer la tendance observée.

Une analyse génomique des souches humaines et alimentaires a été réalisée afin d'obtenir une structure de la population des *Listeria monocytogenes* et de leur virulence.

L'AQR générique proposée dans le projet d'avis scientifique se base, entre autres, sur les données de contamination issues de l'enquête de référence européenne (2010-2011) (EFSA 2013 et 2014) sur la prévalence de *Listeria monocytogenes* dans les produits prêts à être consommés (RTE Food en anglais) dans trois matrices : les poissons prêts à être consommés (poisson fumé à froid ou à chaud), les produits à base de viande prêts à être consommés (cuits et emballés) et les fromages à pâte molle ou à pâte semi-ferme prêts à être consommés (le fromage frais est exclu).

Les résultats de l'AQR sont ensuite discutés dans le rapport de l'EFSA en se basant sur les résultats d'études menées dans le cadre du même projet, portant sur les caractéristiques génomiques de souches de *Listeria monocytogenes* isolées en Europe et sur des résultats de plusieurs modèles d'attribution des sources.

Malgré cette étude approfondie, la tendance à l'augmentation du nombre de cas de listériose humaine en Europe reste en majeure partie inexpliquée, notamment pour les femmes entre 25 et 44 ans.

Le Groupe scientifique BIOHAZ de l'EFSA identifie le facteur « proportion croissante des populations âgées et des populations sensibles (sauf pour les femmes de 25 à 44 ans) » comme facteur vraisemblablement responsable de l'augmentation du nombre de cas. D'autre part, il

identifie la proportion de personnes sensibles dans la classe d'âge de plus de 45 ans (hommes et femmes) comme le facteur principal qui pourrait expliquer l'augmentation de l'incidence constatée.

L'analyse des données (Lignes 167-169) suggère que la plupart des cas de listériose sont liés à l'ingestion d'aliments prêts à être consommés, contaminés avec des concentrations moyennes à fortes ($10^{3,5}$ - $10^{7,5}$ UFC/portion) de *Listeria monocytogenes*. Selon le rapport, 92% des cas de listériose sont attribuables à des doses supérieures à 10^5 UFC/portion ce qui correspond à des aliments prêts à être consommés avec une concentration supérieures à 2000 UFC/g de *Listeria monocytogenes* au moment de la consommation.

Pour l'ensemble des parties de l'analyse de l'EFSA, le CES BIORISK a formulé des demandes de justifications/argumentations sur les choix et hypothèses posées (choix des matrices étudiées, choix d'interprétation, modalité de validation des résultats du modèle, choix des facteurs étudiés, etc.). Des propositions de reformulations et de clarifications ont été ajoutées.

La limite principale identifiée suite à l'expertise collective de l'Anses est la non-disponibilité et l'hétérogénéité de la qualité des données spécifiques à l'échelle européenne (données socio-économiques, données de contamination, données de consommation par catégorie de population, données sur la préparation/conservation des repas, etc.) pour répondre aux questions posées. De nombreuses sources d'incertitude peuvent entraîner une interprétation erronée de l'impact de certains facteurs pris en compte dans le modèle d'AQR.

D'autre part, le fait que les travaux se basent sur uniquement trois catégories de produits prêts à être consommés constitue une limite importante à l'interprétation des résultats.

Enfin, l'analyse a été menée par classes d'âge et de sexe pour les populations sensibles sans prendre le facteur « Etat membre » comme co-variable, faute de données disponibles. Cette restriction constitue une limite importante à l'interprétation des résultats de l'étude, du fait notamment de la diversité des systèmes d'inspection et de surveillance en Europe. Egalement, une étude de phylogéographie de *Listeria monocytogenes* aurait pu être établie, identifiant des pratiques alimentaires ou des sources propres à certains pays ou groupes de pays européens.

L'impact de ces résultats (en intégrant les limites et incertitudes liées à l'étude) sur le critère de sécurité microbiologique actuel de 100 UFC/g ainsi que sur les analyses à la date limite de consommation aurait mérité d'être discuté par le groupe scientifique européen. De plus, aucune discussion n'est proposée sur les difficultés d'interprétation auxquelles pourraient être confrontés les opérateurs agro-alimentaires à la lecture des résultats présentés dans l'avis.

3.2. Conclusions du CES BIORISK

Le CES BIORISK souhaite tout d'abord souligner le haut niveau d'approfondissement et la rigueur des travaux présentés dans le projet d'avis de l'EFSA. Bien que nombreuses, les limites de l'étude sont exposées et discutées assurant ainsi transparence et bonne compréhension de la démarche européenne.

Le CES BIORISK émet les conclusions et recommandations suivantes :

- concernant la prise en compte de l'hétérogénéité des systèmes de détection et de surveillance et des pratiques de consommateurs, il aurait été intéressant de mener une analyse par clusters géographiques, par exemple. La déclinaison des modèles avec des données plus complètes et dans un nombre limité d'Etats membres serait plus pertinente au regard de la problématique,

- si l'on considère la large circulation de *Listeria monocytogenes* depuis ses réservoirs animaux et environnementaux jusqu'au consommateur, il serait opportun d'élargir les analyses au-delà des aliments prêts à être consommés, seule catégorie étudiée dans le document,

Pour conclure et après avoir acquis des données complémentaires, un nouveau travail mériterait d'être entrepris pour aboutir à des recommandations de gestion concrètes au niveau européen. Afin de réduire le niveau d'incertitude et permettre une analyse, il est suggéré de mener une réflexion sur les données à acquérir et les méthodes à utiliser pour expliquer de manière convaincante l'augmentation de l'incidence de la listériose humaine en Europe.

En France, un bilan des données disponibles pour l'attribution des sources (Anses 2017) a été établi dans ce sens.

4. CONCLUSIONS ET RECOMMANDATIONS DE L'AGENCE

L'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail endosse les conclusions du CES BIORISK.

Dr Roger Genet

MOTS-CLES

Listeria monocytogenes, aliments prêts à être consommés, listériose humaine, analyses de séries temporelles

Listeria monocytogenes, ready-to-eat food products, human listeriosis, time series analysis

BIBLIOGRAPHIE

- Allerberger F. and M. Wagner. 2010. Listeriosis: a resurgent foodborne infection. *Clin Microbiol Infect* 16: 16-23
- Anses. 2017. " Avis et rapport de l'Anses du 28 juin 2017 relatif à l'attribution des sources des maladies infectieuses d'origine alimentaire"
- Charlier, C., Perrodeau, E., Leclercq, A., Cazenave, B., Pilmis, B., Henry, B., Lopes, A., Maury, M. M., Moura, A., Goffinet, F., Dieye, H. B., Thouvenot, P., Ungeheuer, M. N., Tourdjman, M., Goulet, V., de Valk, H., Lortholary, O., Ravaut, P., Lecuit, M., & group, M. s. 2017. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. *Lancet Infect Dis* 17(5) : 510-519
- Chen Y, WH. Ross, VN. Scott, DE. Gombas. 2003 . *Listeria monocytogenes*: Low levels equal low risk. *Journal of Food Protection* 66(4):570-577
- Derens-Bertheau, E.; Osswald, V.; Laguerre, O.; Alvarez, G. 2014. Chain of chilled food in France. *Int.J. refrig* 52:161:167. doi:10.1016/j.ijrefrig.2014.06.012
- Duret , S., L. Guillier, H-M. Hoang, D. Flick, O.Laguerre. 2014. Identification of the significant factors in food safety using global sensitivity analysis and the accept-and-reject algorithm: application to the cold chain of ham. *International Journal of Food Microbiology* 180: 39-48
- European Centre for Disease Prevention and Control. 2016. Annual Epidemiological Report 2016 – Listeriosis. Internet Stockholm: ECDC
- Ellouze, M., Gauchi, J.-P. and Augustin, J.-C..2010. Global Sensitivity Analysis Applied to a Contamination Assessment Model of *Listeria monocytogenes* in Cold Smoked Salmon at Consumption. *Risk Analysis*, 30: 841–852
- European Food Safety Authority. 2013. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat (RTE) foods in the EU, 2010–2011 Part A: *Listeria monocytogenes* prevalence estimates. *EFSA Journal* 11(6):3241, 75 pp. doi:10.2903/j.efsa.2013.3241
- European Food Safety Authority. 2014. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat foods in the EU, 2010-2011. Part B: analysis of factors related to prevalence and exploring compliance. *EFSA Journal* 12(8):3810, 73 pp. doi:10.2903/j.efsa.2014.3810
- Lardeux A.-L., L. Guillier, E. Brasseur, C. Doux, J. Gautier and N. Gnanou-Besse. 2014. Impact of the contamination level and the background flora on the growth of *Listeria monocytogenes* in ready to-eat diced poultry. *Applied Microbiology* 60: 481-490

- Leclercq, A., Chenal-Francisque, V., Dieye, H., Cantinelli, T., Drali, R., Brisse, S., & Lecuit, M. 2011. Characterization of the novel *Listeria monocytogenes* PCR serogrouping profile IVb-v1. *Int J Food Microbiol* 147(1):74-7
- Maertens de Noordhout, C., Devleeschauwer, B., Angulo, F. J., Verbeke, G., Haagsma, J., Kirk, M., Havelaar, A., & Speybroeck, N.. 2014. The global burden of listeriosis: a systematic review and meta-analysis. *Lancet Infect Dis* 14(11): 1073-1082.
- Maury MM, Tsai YH, Charlier C, Touchon M, Chenal-Francisque V, Leclercq A, Criscuolo A, Gaultier C, Roussel S, Brisabois A, Disson O, Rocha EP, Brisse S, Lecuit M. 2016. Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. *Nat Genet* 48:308-313.
- Maury MM, Chenal-Francisque V, Bracq-Dieye H, Han L, Leclercq A, Vales G, Moura A, Gouin E, Scotti M, Disson O, Vázquez-Boland JA, Lecuit M. 2017. Spontaneous virulence loss in natural populations of *Listeria monocytogenes*. *Infect Immun*. doi:10.1128/IAI.00541-17
- Moura, A., A., Criscuolo, A., Pouseele, H., Maury, M. M., Leclercq, A., Tarr, C., Bjorkman, J. T., Dallman, T., Reimer, A., Enouf, V., Larssonneur, E., Carleton, H., Bracq-Dieye, H., Katz, L. S., Jones, L., Touchon, M., Tourdjman, M., Walker, M., Stroika, S., Cantinelli, T., Chenal-Francisque, V., Kucerova, Z., Rocha, E. P., Nadon, C., Grant, K., Nielsen, E. M., Pot, B., Gerner-Smidt, P., Lecuit, M., & Brisse, S. 2016. Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. *Nat Microbiol*, 2: 16185
- Moura A, Tourdjman M, Leclercq A, Hamelin E, Laurent E, Fredriksen N, et al. 2017. Real-time whole-genome sequencing for surveillance of *Listeria monocytogenes*, France. *Emerg Infect Dis* 23(9): 1462-1470. Doi:10.201/eid2309.170336
- Pohl A.M., R. Pouillot and J Van Doren . 2017. Changing US Population Demographics: What Does This Mean for Listeriosis Incidence and Exposure? *Foodborne Pathogens and Disease* 14(9): 524-530
- Pouillot, R., K. Hoelzer, Y. Chen, S. Dennis. 2015. *Listeria monocytogenes* dose response Revisited-incorporating adjustments for Variability in Strain Virulence and Host Susceptibility. *Risk analysis* 35: 90-108
- Ragon M, Wirth T, Hollandt F, Lavenir R, Lecuit M, Le Monnier A, et al. 2008. A New Perspective on *Listeria monocytogenes* Evolution. *PLoS Pathog* 4(9):e1000146. Doi :10.1371/journal.ppat.1000146

ANNEXE 1 : PRESENTATION DES INTERVENANTS

PRÉAMBULE : Les experts membres de comités d'experts spécialisés, de groupes de travail ou désignés rapporteurs sont tous nommés à titre personnel, *intuitu personae*, et ne représentent pas leur organisme d'appartenance.

RAPPORTEURS

M. Frédéric CARLIN – INRA. Microbiologie des aliments, filière fruits et légumes, technologie de décontamination

M. Philippe FRAVALO – Université de Montréal. Hygiène et microbiologie des aliments (viandes et produits carnés)

M. Laurent GUILLIER – Anses, Laboratoire de sécurité des aliments. Modélisation, appréciation quantitative des risques, microbiologie des aliments

M. Régis POUILLOT - Modélisation, appréciation quantitative des risques, microbiologie des aliments

COMITÉ D'EXPERTS SPÉCIALISÉ

■ CES « Evaluation des risques biologiques des aliments » (BIORISK)

Président

Mme Isabelle VILLENA – CHU Reims. Parasitologie, infectiologie

Membres

M. Jean-Christophe AUGUSTIN – Ecole nationale vétérinaire d'Alfort. Modélisation, appréciation quantitative des risques, microbiologie des aliments

Mme Anne BRISABOIS – Anses, Laboratoire de sécurité des aliments. Microbiologie des aliments, écologie microbienne, méthodes analytiques

M. Frédéric CARLIN – INRA. Microbiologie des aliments, filière fruits et légumes, technologie de décontamination

M. Olivier CERF – Professeur émérite. Ecole nationale vétérinaire d'Alfort. Evaluation des risques microbiologiques, microbiologie des aliments

M. Pierre COLIN – Professeur émérite. Université de Bretagne Occidentale. Hygiène et microbiologie des aliments (viandes et produits carnés – volailles)

M. Philippe DANTIGNY – AgroSup Dijon. Mycologie, procédés de décontamination, écologie microbienne

Mme Florence DUBOIS-BRISSONNET – AgroParisTech. Microbiologie des aliments, mécanismes d'adaptation au stress, biofilms, hygiène des surfaces et des procédés

M. Michel FEDERIGHI-ONIRIS, Nantes - Hygiène et microbiologie des aliments (viandes et produits carnés), procédés de décontamination

M. Benoit FOLIGNE – Faculté de médecine de Lille 2. Microbiote intestinal, interaction écosystème alimentaire/microbiote

Mme Florence FORGET-RICHARD – INRA. Mycotoxines, champignons filamenteux, biochimie, filières céréales

M. Philippe FRAVALO – Université de Montréal. Hygiène et microbiologie des aliments (viandes et produits carnés)

M. Pascal GARRY – Ifremer, Nantes. Hygiène et microbiologie des aliments (viandes et produits carnés, coquillages)

M. Michel GAUTIER – Agrocampus Ouest. Microbiologie des aliments, biologie moléculaire, génie génétique

M. Laurent GUILLIER – Anses, Laboratoire de sécurité des aliments. Modélisation, appréciation quantitative des risques, microbiologie des aliments

Mme Nathalie JOURDAN-DA SILVA – Santé publique France. Epidémiologie des maladies entériques et zoonoses

M. Alexandre LECLERCQ – Institut Pasteur. Microbiologie des aliments (*Listeria monocytogenes*, *Yersinia* entéropathogènes), méthodes phénotypiques et moléculaires

M. Simon LE HELLO – Institut Pasteur. *Salmonella*, épidémiologie, méthodes phénotypiques et moléculaires

M. Eric OSWALD – CHU Toulouse. Infectiologie clinique, écologie Microbienne, *E. coli*

Mme Nicole PAVIO – Anses, Laboratoire de santé animale de Maisons-Alfort. Virologie

Mme Sabine SCHORR-GALINDO – Université Montpellier 2. Mycologie, écologie microbienne

Mme Muriel THOMAS – INRA. Microbiote intestinal, probiotiques

PARTICIPATION ANSES

Coordination scientifique

Mme Diane CUZZUCOLI – Chargée de projets scientifiques et techniques – Unité d'évaluation des risques liés aux aliments (UERALIM) – Direction de l'évaluation des risques

Secrétariat administratif

Mme Catherine FRANCOIS – Anses – Direction de l'Evaluation des Risques

ANNEXE 2 : COMMENTAIRES TRANSMIS A L'EFSA

1. Commentaires sur le résumé: *Summary*

Line 102-104: The invasive forms have undeniably the heaviest public health impact. But no information is available on the non-invasive cases associated to a non-harmless clinical picture (flu syndrome, digestive symptoms) which certainly also impact on the public health. Gastroenteritis is now well established for Lm infection but are widely underestimated and reported. Some management of gastroenteritis could avoid its evolution to invasive forms, as suggested by Allerberger (2010).

Line 115: Please specify with: "are rarely isolated from *invasive form* clinical samples" (indeed only invasive form are investigated).

Lines 135-137: Why is ice-cream included here? (Plant-derived origin and not RTE milk derived products). Please argue the use of the term "unexpected" or reformulate.

Line 157: Reporting minimum and maximum values of a series is not meaningful here.

Lines 159-160: "Since the majority of studies of food handling are from few countries only, this may lead to some uncertainty": please precise or rephrase. Suggestion: "There is an uncertainty on the actual distribution in the EU because the studies were developed in few countries only".

Lines 167-169 and 218-220: May you precise and discuss the impact on security microbiological criteria for Lm in EC 2073/2015 regulation, on analysis at shelf life of the products and possible interpretation of this data by food operators.

Lines 176-178: How do these QMRA results align with observations?

Lines 179-181: What about other food?

Line 182: "median number of cases" What is the corresponding distribution? What is the uncertainty? Please rephrase or precise somewhere in the report if it is variability distribution or uncertainty distribution.

Line 219: We understand that $10^5/50$ is 2000. However, the "average" (arithmetic mean) concentration in RTE that causes listeriosis is probably much higher than 2000 CFU/g. Few servings with 10^8 , 10^9 CFU would shift this mean. The sentence would be more correct if written "Assuming an average serving size of 50 g, this would correspond to an average *L. monocytogenes* concentration in RTE foods above 2,000 CFU/g at the time of consumption".

Line 231: Suggestion: The increasing average age of the first pregnancy could lead to an increase of near-40 year old pregnant woman proportion, for whom the susceptibility could be different.

2. Commentaires sur la partie 1 du rapport : 1. Introduction

➤ Transmission routes of *L. monocytogenes* in RTE foods

Lines 427-428: Add the prevalence: "the higher the pathogen concentration and the higher the prevalence is, the more effective the control processes need to be in order to reduce concentrations"

Lines 436-437: Inhibition of growth does not reduce microbial load.

Lines 443-445: *L. monocytogenes* is able to form biofilm. But in the given example (=cutting and slicing), *L. monocytogenes* is transferred on surfaces with other micro-organisms and food materials. Rephrase with "*L. monocytogenes* can adapt to (and persist in) a biofilm environment in addition to its ability to form biofilms" (maybe more adapted to the given examples).

Line 446: Please remove "sanitisers" (sanitizers are disinfectants).

Figure 1: Suggestion: Add an arrow from "raw material" to "Domestic environment".

Line 472: Sources may also be environmental. Furthermore, raw products were not taken into account here.

Line 494: There are other reasons than bacteriocin productions, simple competition with LAB (Jameson effect) can be another pertinent one (e.g. Lardeux et al, 2015).

Line 496: Differences between strains do exist (Ariany et al. papers, Koutsoumanis review on intraspecific variability) but the variability of parameters values has never been pointed out in exposure/risk assessment as a major source of variability compared to other sources. See papers that conducted a sensitivity analysis (Ellouze et al, 2010; Duret et al, 2014).

Lack of regional study

The Panel missed an opportunity to explore regional variations in the incidence of listeriosis. Without considering each country independently, a simple regionalization (2-4 regions, according to typical diets) might have provided some interesting clues to explain the pattern of listeriosis cases. Demographics are also various in these countries and a comparison could help the purpose.

Line 498: It would be interesting to highlight that monitoring and sanitary programs are very heterogeneous across European member states.

Line 526: Does these guidelines on sampling (or other guideline) include trend analyses on product's contamination or environmental contamination?

Line 539: It may be noted that it is possible to rate the virulence (InIA sequencing or CC setting).

3. Commentaires sur la partie 2 du rapport : 2.Data and methodologies

➤ ECDC data on cases of human listeriosis

Line 670: You don't refer to this WHO Global burden study and discuss it against your data (Martens 2014).

Lines 677-678: later in the paper, Germany is taken into account (and not Belgium) as mentioned here. How do you explain the coverage improvement for Belgium/Germany (mentioned later)?

Lines 687-689: The fact that cases under one year old were excluded from the TSA because they were mainly related to pregnancies is not correct according to international publication on the subject. It is recognized that child with an age less than 28 days or 1 month is a pair with the mother and was counted as one case. After this period 1 month, it becomes two cases. If not, e.g. you introduce a bias on nosocomial contamination in hospital or eat of specific food possibly contaminated combined to infant food formulae. A sentence shall be put to modulate the report on this point.

Lines 698-701: The described PCR serogroup is wrong for IIb because IIb is serovar 1/2b and 3b. The scheme is supplemented by WHOCC with IVb-V1 so refer to Leclercq et al., 2011. In the scheme of Doumith the PCR group L missing so all other serovars not described before. Please, correct the PCR group according to Doumith et Leclercq publication (Leclercq 2011).

Lines 702-706: Not take in account IIc and IIb is not scientifically correct. We need to know where we are with this last two PCR serogroup. It is a bias in the analysis.

➤ Eurostat data on European demographic statistics

Line 755: "The prevalence of pregnant women": please rephrase, pregnancy is not a disease (suggestion: "proportion of pregnant women").

➤ EU-wide baseline survey data

Line 781: Could you remind briefly how the samples were chosen (was it random sampling?) and what was the size of each tested sample (25 grams?, more?)?

➤ EFSA monitoring data

Lines 790-791: analytical methods are harmonized as it is an EN ISO 11290-1/A1 standard and validated by a European project SMT4 from European Commission in 2000 so "to a certain extent" means that you have an analytical bias that impact directly your estimation of CFU/serving value and the target of EURL is to

harmonize in Europe the analytical methods so it is a direct critic of its work. Please rephrase “to a certain extent”

Lines 806-819: This paragraph implies issues with quantitative interpretation in this study: a considerable proportion of detection in 25g of food will not be taken into account quantitatively or can be assigned to 10 ufc/g (while a positive detection can concern a large quantity = 10^6 ufc/g).

Limitation to three RTE food.

The whole report uses the very strong assumption that all cases of listeriosis in the European Union are linked to three types of RTE only. This assumption is acknowledged, but its limitation should be thoroughly discussed in a report that looks for some clues in the increase of the listeriosis incidence rates, notably after the observation of “unexpected” sources of *Listeria* in the USA (produce, notably).

The observation of outbreaks linked to complex foods (sandwiches), vegetable/fruits and the new trends of consumption observed in the EU (more RTE) should trigger some discussions regarding these potential sources. The hypothesis of an increase in the listeriosis incidence linked to food other than the “usual suspects” should be considered.

➤ Data from scientific literature and outsourcing activities

Line 873: Why are the in-house method evaluation excluded from the study? Indeed, the reference method is often specified in those studies and sampling methods are not systematically biased.

➤ EFSA Consumption data on RTE foods

Line 910: Please specify the total number of studies by EU member state (to specify the distribution of studies).

➤ Surveillance of human listeriosis

Lines 956-963 and Lines 982-995: French NRC for *Listeria* and Sante Publique France were participants (member of FWD ECDC WG) by providing metadata: microbiological/epidemiological data but were not referred in the acknowledgments of this report.

➤ Time series analysis of human listeriosis trends,2008-2015

Line 988-994: Not sure that the fact that the dataset is in a “wide” or “long” shape is of interest for the reader.

➤ Generic QMRA model

Line 1193: The distributions used a maximum of 6.1 log₁₀ CFU/g. This parameter is very important (see sensitivity analysis). It might have been taken from Pouillot et al (2015), who took it from Chen et al (2003). You should provide the reference for this value; discuss it and how it aligns with the observations of the baseline survey data or the exposure model from the QRA.

Line 1196: The choice of this strong assumption should be more discussed.

Figures 4 and 5: Figure 5 should include the empirical cumulative distribution function (ECDF) from figure 4 for a better comparison. Or, alternatively, Figure 4 should have the modeled CDF overplotted so that we can check the good fit of the beta generalized models to the data.

Line 1232: The choice of this strong assumption should be more discussed.

Line 1250: The T_{min} value (-1.18°C) originates from FDA-FSIS 2003. Please refer to this report.

Line 1251: The EGR should be set to 0 when T < T_{min}.

Line 1253: In the R- code (Line 6304 Appendix C of the document) the temperature is truncated to -2°C and 15°C. Please specify it here.

Line 1258: This is the Rosso model. Please refer to it. Also, there is a “1 + “missing in the denominator (the denominator is OK in the R code line 6252).

Line 1260: “This primary growth model does not consider a lag time”. No justification for this assumption?

Line 1287: Please precise the Unit of C(t)

Figure 6: Precise and rephrase legend and title of Y-axis.

Line 1326: Re-Specify here what TEO means (total number of eating occasions).

Line 1332: The Pouillot et al (2015) dose-response model uses a standard deviation of the log-normal distribution of 1.62 because they consider "homogeneous" populations (e.g. transplant individuals, pregnant women, see Table 12). By considering more heterogeneous populations (example: Male ≥ 75 yo category include individuals in good shape and individuals with severe underlying conditions), the assessors should reconsider and evaluate their own parameter.

Line 1339: It should be mentioned that the model is actually scaled to the number of cases.

4. Commentaires sur la partie 3 du rapport : 3. Assessment

➤ Introduction to the species of *L. monocytogenes*

Line 1378: Some new species have been identified in primary production samples and in food processing environment. For *L. fleischmannii*, see den Bakker et al., 2013 and Bertsch et al., 2013. Pour *L. newyorkensis* and *L. booriae* see Weller et al., 2015. For *L. rocourtiae*, it comes from lettuce (Leclercq et al., 2009)

Line 1378: You have to complete your sentence to underline the rare case of *L. ivanovii* contamination that it becomes a reality and some foods are heavy contaminated.

➤ Epidemiology of human listeriosis in the EU/EEA

Line 1411: The exhaustibility and reliability of data that support the sentence "most-travel-related cases have acquired the infection within the EU/EEA" are not really present and this sentence has a great impact for image of Europe outside.

Line 1417: The sensitivity of the surveillance system was estimated at 83% by capture-recapture studies in France (Goulet, 2001).

➤ Pregnancy associated human listeriosis cases

Lines 1470-1475: This is unclear. Are the values reported only for the cases for which you had the information regarding pregnancy? What could be the bias, if any, of this under reporting?

Table 5: There might be a typo for % in the 15-24 year old group in 2010 (14.3% seems too low).

Lines 1467-1480 and lines 1885-1941 and lines 4163-4165: The largest reported cohort in the literature has been published by France where an exhaustive system of surveillance exists for human cases. May you compare your results at European level with the study of Charlier et al. 2017 ?

➤ Reported food-borne listeriosis outbreaks

Line 1492: Leaving 41% of the outbreaks linked to foods that are not considered at all in this report.

Line 1530: This caramel apple outbreak is also interesting as young healthy children got listeriosis.

Lines 1530-1536: More cases (10) were observed in this outbreak (see CDC <https://www.cdc.gov/listeria/outbreaks/ice-cream-03-15/index.html>).

Epidemiological relationship between *L. monocytogenes* isolates of human and food origin along the food chain

Lines 1486-87: first may you complete *Listeria* with *monocytogenes*. Please correct in other part in the report.

A drawback of the chapter is the definition of outbreaks and food alerts that you not clearly defined and studied. Moreover, the human cluster is not really defined: it is really important for Lm to have this terms and categories defined and studied.

Line 1582: Could you say one word about the sampling of the strains (representativeness), notably for the food isolates? More generally, for all these studies regarding comparison of clinical and food strains, please provide a better description and discussion of the representativeness of the samples. If the sample is not representative of *Listeria* in food, the report of percentage is meaningless.

Line 1588: May you refer to cgMLST scheme used as it is not the Moller Nielsen scheme but perhaps the one describe in Moura et al. 2016.

Line 1594: What about other sources (Non animal sources)?

➤ Analysis of RASFF :

One point that we want to see with RASFF is the efficacy of the system so in how many RASFF cases we could detect human cases associated with reported foods in Europe.

➤ Summarising remarks for hazard identification :

Lines 1646-1648: It is immunocompetent people or no?

➤ Biology and virulence of Lm :

Lines 1716-1739: Moura et al., 2016 have also showed some of the findings that you underlined. Please refer.

Line 1703: for gastroenteritis, please refer to Ooi and Lorber, 2005 in Clinical practice

➤ Hypothesis of QMRA

Line 1638: Was the evolution of “pregnant 15 to 24 year old woman “ reported proportion between 2008 and 2015 taken into account ? Indeed, this proportion decreases since 2008.

Line 1682: Do you mean food (rather than food-borne) isolates?

➤ Virulence variability in *L.monocytogenes*

Line 1784: Could you recall and discuss briefly how the strains were obtained in this study, representativeness? Indeed, a large sample doesn't make it representative.

Line 1806: Avoid “food-borne” origin. Prefer “food origin”.

The results rely on the hypothesis that the distribution of virulence of the strains is the same between the different food categories. Although some strains/clonal complexes are equally distributed between the different food categories, small differences in relative percentages of high/medium/low virulent clonal complexes could lead to huge differences (because most listeriosis are linked to virulent clonal complexes).

➤ Clinical picture of reported human listeriosis cases in the EU

Line 1893: Data are available for only 39.2% of cases. Could we expect a bias (more info for specific clinical picture) or should we consider that these data miss at random?

➤ Growth models

Sophisticated models exist for growth rate prediction. It would have been of interest to show comparison for some of the 13 categories of exponential growth rate (5°C) and these models.

➤ Dose response

The r values have been inferred for each of the 14 categories of defined population. The status of population (cancer, HIV, etc.) is not taken into account. Wouldn't have been possible to define age/health status category?

Line 1982: FDA – FSIS (2003) used the exponential dose response only for mice models. The human model is much more complex than that.

Line 2003: Note that the outcome of the FDA FSIS 2003 model was death (to be compared with other models, considering invasive listeriosis).

Line 2020: The FDA/FSIS 2003 should not be considered as an exponential model.

➤ Evidence for exposure assessment

Persistence of strains in the food processing environment

Lines 2151-2154: Applicable for the “raw products sector” but not always relevant for the “cooked products sector” (including RTE Food).

Line 2172: Precise what does “tolerate” means?

Line 2242: To be mentioned here: the question of definition between persistence vs recurrence remains. Regular contamination by the same strain from the primary production can lead to an inaccurate status of *L. monocytogenes*.

➤ Evidence for risk characterisation

Lines 3151-3156: Vegetables should be an iceberg in terms of knowledge of contamination by Lm and consumer eat more raw vegetables (and fruits) with direct transmission from producers and biological culture. This paragraph underlined that this report gave no more additional data. Study shall be conducted on this topic.

EFSA monitoring data

Figure 11: Specify when it is at the end of processing or during the processing. Mention "During cutting plant" is not specified in any food types (x-axis).

Prevalence of *L.monocytogenes* in RTE Foods from literature studies

Figure 12: The use of the box plot should be considered with caution as the sample size (number of samples per study) and the sampling design vary from one study to the other.

Figure 13: Incomplete unit for x-axis.

Consumer food handling

Line 2542: Assumption potentially inaccurate: thermal profile of a can of Rillettes for example is different: it does not only depend on time and temperature in fridge (but also of the exits frequency from the fridge for example).

Table 16: The work of Derens-Bertheau et al. 2014 could be also cited.

Factors impacting the prevalence and concentration of *L.monocytogenes* in RTE foods

Lines 2718-2755: please provide confidence intervals around the OR.

Temperature of domestic refrigerators

Lines 2990-2995: The authors of this publication proposed two distributions for describing temperature distribution (one for North EU one for South). Yet, their data analysis didn't prove this.

➤ Results from the review of QMRA outputs

Line 3090: Please, update the review with the reference FDA-FSIS, 2013 (notably on the potential contamination of the environment from products that do not support growth to products that support growth).

Line 3140: Williams et al, 2009 paper is not on that subject.

Lines 3182-3205 (and in the whole section): Can you explain what are the intervals presented lines 3184-3185? Do they represent uncertainty (serving to serving variability being integrated), variability, or a mix of both?

If it is "mostly variability" as indicated line 3195: what variability is considered (it can't be serving to serving as the lower bound should be 0 to account for non-contaminated products).

Line 3207 and further: Please specify for the "median": in what dimension. Appendix I should provide some more details.

Line 3237: Is the dose response model calibrated to provide the observed number of cases? If yes, it should be reminded.

Line 3333: Remind that these numbers reflects the strong assumption that all cases are linked to these three RTE foods in EU.

Lines 3448-3450: There is probably an over-interpretation about potential factors here, in the light of the concerned population size (EU level).

➤ Evaluation of factors that may explain the epidemiological trend of human listeriosis

Line 3589: It is expected because the dose response model is scaled to the epidemiological data: it should be reminded to the readers.

Lines 3614-3618: it is important to discuss here about the relevance of the option 3 (use US contamination data).

Lines 3716 and further: Provide the results either in log or not in log, but not a mix.

Line 3823 and Figure line 3828: it is “genoserotype or PCR group” but not “serotype/serogroup” that are obtained by agglutination of antifactor sera with antigens.

➤ Uncertainty analysis of the gQMRA model

Line 3919: Because the DR model is scaled to the epidemiological data, the absolute number predictions have little interest here. Suggestion: remove this sentence.

➤ Synthesis of evidence of factors

Line 3998: It might have been clearer to provide the expected evolution of the number of listeriosis cases from the change in demographics, had the incidence rates been stable from 2008 to 2014, and to compare those numbers with the observed cases (see Pohl et al. 2017).

Line 4026: Actually, QMRA are sensitive to maximum population density (MDP) because, with the current dose responses, the expected number of cases are proportional to the arithmetic mean concentration in food, this mean being highly sensitive to the MPD. But do we expect a change in the MPDs?

Line 4079: No year for Thomas et al (specify 2013)

➤ Conclusion of factors contributing to the human listeriosis trend in the EU

Line 4104-4105: See previous comment. Doses $> 10^5$ CFU/g do not lead to a mean concentration in RTE food equal to 2000 CFU/g.

5. Commentaires sur les parties 4 et 5 du rapport : 4. Conclusions -5. Recommendations

The outsourcing activities related to genomics (Moller-Nielsen et al, 2017) revealed that epidemiological investigations conducted at European level using whole genome sequencing (WGS) would permit to identify the origin of listeriosis sporadic cases. Efficient epidemiological investigation can also be seen as a way to reduce risk. Identifying the food origin even outside the context of an outbreak will prevent new sporadic cases if an action is taken in the food industry potentially related to the first sporadic case.

As high concentrations/doses (>2000 cfu) are expected to explain the most listeriosis cases, one could expect strong recommendations on shelf-life determination. Most RTE food shelf-lives are not determined based on *Listeria monocytogenes* behavior within the RTE. The determination of growth potentials, as a way to simply assess if the shelf-life is appropriate should be recommended.

➤ General comments on recommendations

Despite an exhaustive report, there is still a lack of understanding about the increase in the listeriosis incidence rate in the EU.

However, the recommendations provided at the end of the report (less than half a page, limited to five bullet points) will probably not help to have a better understanding of listeriosis in the future. Other in-depth recommendations should be written, including, e.g., more work on source attribution (coupled with next-generation sequencing (NGS), better epidemiological record (including a better understanding on the impact of comorbidities as well as other factors (demographic, sociological, etc.)), prevalence (not limited to “out of compliance”) studies beyond the three classical RTE food (with enumeration, NGS, etc. ...

The charge of the Panel BIOHAZ should include recommendations to the Member States, but also to the research community.

6. Commentaires sur les annexes / Appendix

➤ Appendix B

Figure 32: What information would you get from the seasonality trend? Please discuss in the text.

➤ Appendix C

Table 33: Why are the max for Smoked fish and Cheese set to 5 and 7? Also shouldn't the other be 6.1 (in accordance with the text, the footnote, and with Chen et al, 2003)?

Table 34: What are the parameters “shift”. Did you fit a shifted log-normal distribution? If yes, it is not implemented in the code (Line 6294).

Table 37: Please provide some explanations as a foot note regarding the parameters RefSdLog and RefSdLogl.

➤ R Code

Please review multiple typos in the comments.

Line 6154: Script1.R would need some libraries loaded in Script2.R (e.g. tidyverse for the %>% function).

Line 6155-6177: Please, acknowledge or refer to Pouillot et al, 2015.

Line 6308: There is an error here: the formula should set EGRr to 0 when $T < T_{min}$.

➤ Appendix F

Table 40: it is reduced PI-PLC activity. You can also now put hly from Maury et al. 2017

Source missing : Ragon et al., 2008, Maury et al., 2016 and Moura et al., 2017