Exposure assessment of consumers to Listeria monocytogenes related to the consumption of non-prepackaged ready-to-eat cooked meat products

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OBJECTIVE

- To assess the risk of listeriosis related with the consumption of non-packed RTE cooked meat products sliced at retail food service environments in Greece.
- To identify and propose mitigation strategies for reducing the risk of listeriosis related with the consumption of non-packed RTE foods

EFET
HELLENIC FOOD AUTHORITY

efsa

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RISK ASSESSMENT INFOGRAPH

PREDICTIVE MICROBIOLOGY

Environmental data for the food chain
(time, temperature, pH, aw …)

DATA REQUIREMENTS

- Prevalence and concentration of Lm at starting point (time of slicing)
- Product characteristics affecting growth of Lm (pH, aw, nitrites, LAB)
- Time-Temperature data for domestic storage
- Accurate model predicting growth during domestic storage
- Serving size
- Dose response relationship (for normal and susceptible populations)
- Annual number of servings (for normal and susceptible populations)

PREVALENCE AND CONCENTRATION OF LM AT STARTING POINT

- Positive samples of swabs of the entire exposed area of the cutting tool used for slicing of the non-prepackaged RTE cooked meat product were identified in **2 out of the 300** samples (**0.67%**).
- In only **one of the 2 cases**, where the swab tested positive for L. monocytogenes, was the microorganism also detected on the meat product previously sliced (**0.33%**)
- Concentration below the limit of quantification (LOQ:10 cfu/gr)

PRODUCT CHARACTERISTICS AFFECTING LM GROWTH

- In total **87 products** with 4 replicates for each product were analyzed for pH, water activity, concentration of nitrites and concentration of the lactic acid bacteria (LAB)
- Products represent >85% of total sales

Database on the physicochemical characteristics (pH, aw, concentration of nitrites) and concentration of lactic acid bacteria of all non-prepackaged RTE cooked meat products sampled from the market

Code	Type of meat product	Type of cessing (boiled or oked or roasted)	Producing company (code)	Retail code	Expiration date	a_w	Water Phase Salt	pH	Nitrite (ppm)	LAB (cfu/g)	LAB (Logcfu/g)
1a	Pariza - Salami	Boiled	1	2	10/7/2018	0.976	4.084	6.36	16.82	113000	5.053
1b	Pariza - Salami	Boiled	1	2	10/7/2018	0.973	4.564	6.33	15.86	43000	4.633
1c	Pariza - Salami	Boiled	1	2	13/3/2018	0.977	3.923	6.26	12.8	110000	5.041
1d	Pariza - Salami	Boiled	1	2	13/3/2018	0.977	3.923	6.28	12.26	130000	5.114
2a	Pork Shoulder	Boiled	1	4	8/1/2017	0.978	3.760	6.62	14.31	2000	3.301
2b	Pork Shoulder	Boiled	1	4	8/1/2017	0.976	4.084	6.6	15.81	1600	3.204
2c	Pork Shoulder	Boiled	1	4	9/4/ 2018	0.978	3.760	6.42	20.32	2700000	6.431
2d	Pork Shoulder	Boiled	1	4	9/4/2018	0.978	3.760	6.43	20.05	2500000	6.398
3a	Mortadella Bologna	Boiled	1	1	8/7/2017	0.98	3.433	6.22	17.28	285000	5.455
3b	Mortadella Bologna	Boiled	1	1	8/7/2017	0.979	3.597	6.3	16.17	191000	5.281
3c	Mortadella Bologna	Boiled	1	3	24/5/2018	0.981	3.269	5.66	3.58	3070000	6.487

PRODUCT CHARACTERISTICS AFFECTING LM GROWTH

- In total **87 products** with 4 replicates for each product were analyzed for pH, water activity, concentration of nitrites and concentration of the lactic acid bacteria (LAB)

Summary statistics for pH, aw, LAB and nitrites of cooked meat products available on the Greek market

	pH	a_w	LAB (log cfu/g)	$NaNO_2$ (ppm)
Mean	6.34	0.979	4.74	26.60
Standard error	0.016	0.000259	0.059	1.84
Median	6.35	0.98	4.7	17.04
Mode	6.35	0.982	4.6	1.4
Standard deviation	0.2908	0.0046	1.05	32.76
Range	1.55	0.028	4.25	207.4
Minimum	5.63	0.965	2.55	0.16
Maximum	7.18	0.993	6.8	207.6

VALIDATION OF PREDICTIVE MODELS FOR LM GROWTH

- The FSSP model was selected as the most appropriate since it includes all parameters affecting growth (Temperature, pH, water activity, concentration of nitrites and concentration of the lactic acid bacteria)

FSSP

DTU
Technical University of Denmark

VALIDATION OF PREDICTIVE MODELS FOR LM GROWTH

- Three RTE meat products (A: boiled turkey, B: boiled ham, C: smoked turkey) were selected representing a high, medium and low concentration of NaNO2.
- Products were inoculated with the strain 154 isolated form the slicing machine and stored at three static (4, 7, 12° C) and two dynamic temperatures simulating temperature conditions during domestic storage
- Observed growth of Lm and LAB was compared to predicted growth by FSSP

VALIDATION OF PREDICTIVE MODELS FOR LM GROWTH

Characteristics of products used for experiments on static temperature at 4°C for 16 days

Characteristics	Product A	Product B	Product C
pH	6.27	6.2	6.1
a_w	0.989	0.985	0.982
$NaNO_2$ (ppm)	46.5	15	0

VALIDATION OF PREDICTIVE MODELS FOR LM GROWTH

Characteristics of products used for experiments on first dynamic scenario at 4oC for 12 hours - 12oC for 12 hours for a repeated cycle of 96 hours

Characteristics	Product A	Product B	Product C
pH	6.13	6.09	5.94
a_w	0.982	0.984	0.982
$NaNO_2$ (ppm)	41.05	28.86	0

VALIDATION OF PREDICTIVE MODELS FOR LM GROWTH

Characteristics of products used for experiments on second dynamic scenario. The incubators temperature was 4oC but for 3 hours every day its door was left open

Characteristics	Product A	Product B	Product C
pH	6.13	6.03	5.93
a_w	0.984	0.988	0.976
$NaNO_2$ (ppm)	40.5	24.2	0

TIME-TEMPERATURE DURING DOMESTIC STORAGE

- 90 domestic refrigerators were surveyed in North Greece. The data loggers were programmed to record the temperature every 15 min for 24 h and were located at the middle shelf of the refrigerator. Temperature data were combined with those from a previous survey (100 domestic refrigerators) presented in Koutsoumanis et al., (2010).

TIME-TEMPERATURE DURING DOMESTIC STORAGE

Statistics of temperature data in domestic refrigerators

Mean temperature of 24 h profiles	
Average	5.97
Standard Error	0.20
Median	6.00
Standard Deviation	2.73
Sample Variance	7.46
Kurtosis	0.78
Skewness	-0.17
Range	15.49
Minimum	-2.40
Maximum	13.09
Count	190

TIME-TEMPERATURE DURING DOMESTIC STORAGE

Length of storage of the meat products

	Usual number of days		Maximum number of days	
	(N)	%	(N)	%
Up to 3 days	124	16	61	8
4-7 days	580	72	539	67
8-10 days	66	8	126	16
11-15 days	12	2	44	5
More than 15 days	3	0	8	1
I don’t know/answer	15	2	23	3

EXPOSURE ASSESSMENT

Prevalence and Concentration of Listeria monocytogenes after slicing at retail.

Prevalence (Ps) of Lm on slicing machines: Beta (3,299)

Assuming a positive slicing machine, prevalence of Lm in the sliced product (Pp): Beta (2,2)

EXPOSURE ASSESSMENT

Prevalence and Concentration of Listeria monocytogenes after slicing at retail.

Overall Prevalence (Ps) of Lm on products (mean 0.5%)

EXPOSURE ASSESSMENT

Prevalence and Concentration of Listeria monocytogenes after slicing at retail.

The number of Lm cells in positive slices (N0) were assumed to follow the Poisson distribution, i.e.

$P_k = P(N_0=k) = \exp(-p) * p^k / k!$ $(k=0,1,2...)$

where the expected value of N0 is $E(N_0)=\rho$. The prevalence can be used to estimate ρ as $p=-\ln(\text{average } 1-P_o)$.

Based on the above description almost 98.5% of positive slices are contaminated with 1 cell of Lm.

EXPOSURE ASSESSMENT

Storage Time at domestic refrigerators

Storage time of RTE meat products at domestic refrigerators was described using a uniform distribution assuming that consumption may occur at any time between the purchase and the end of storage. The minimum value of the uniform distribution was set to zero and the maximum was described with cumulative distribution based on the consumer survey data on storage time.

EXPOSURE ASSESSMENT

Storage Temperature at domestic refrigerators

The temperature data from 190 domestic refrigerators were fitted to various types of probability distributions. The best fitted distribution was the Normal (5.96 , 2.73)

EXPOSURE ASSESSMENT

Growth of Lm during storage at domestic refrigerators

- The Growth of L. monocytogenes was predicted using the validated FSSP model based on the initial concentration of the pathogen and LAB after slicing , the characteristics of the products (pH, aw, nitrite concentration) and the storage temperature and time at domestic refrigerators.
- The maximum population density for both Lm and LAB was set to 8.5 log cfu/g
- In order to describe the variability in the characteristics of the products a triangle distribution was used for each product with the min, max and average value observed in the survey.

EXPOSURE ASSESSMENT

Growth of Lm during storage at domestic refrigerators

- Assuming that LAB contamination is not product specific all data on LAB concentration were described with a cumulative distribution which was used as the initial concentration of LAB for all products.

EXPOSURE ASSESSMENT

Serving size of RTE meat products

- The serving of RTE meat products was described for each product category with a cumulative distribution based on the data collected in the consumer survey . (Figure presents an example of serving size distribution for Mortadella)

EXPOSURE ASSESSMENT

EXPOSURE ASSESSMENT-RESULTS

Example-Lm dose for a parizer product

RISK CHARACTERIZATION-RISK PER SERVING

Dose-Response

The dose-response models of Pouillot et al. (2015) were used. The mathematical structure of the model corresponded with the Exponential model (Eq.1), in which the r parameter is introduced with a LogNormal distribution

P (ill; d, r) = 1-exp(-rd)

Subpopulation	***Parameters log-normal distribution of r***	
	μ(a)	**α(b)**
< 65 years old, no known underlying condition (i.e. "healthy adults")	-14.11	1.62
> 65 years old, no known underlying conditions	-12.83	1.62

(a): Mean.
(b): Standard deviation

f) Risk Characterization

RISK CHARACTERIZATION-RISK PER SERVING

Risk per Serving

Code	Type of meat product	Type of processing (boiled or smoked or roasted)	Producing company (code)	aw	pH	Nitrite	Risk per Serving Consumers <65 years old			Risk per Serving Consumers >65 years old		
							Median	95th Perc.	99th Perc.	Median	95th Perc.	99th Perc.
1	Pariza - Salami	Boiled	A	0.976	6.3	14.4	-15.512	-12.656	-11.352	-14.297	-11.427	-10.110
2	Pork Shoulder	Boiled	A	0.978	6.5	17.6	-15.433	-12.567	-11.242	-14.226	-11.348	-10.007
3	Mortadella Bologna	Boiled	A	0.982	6.0	10.1	-15.457	-12.590	-11.245	-14.255	-11.350	-10.034
4	Turkey toast	Roasted	A	0.982	6.2	9.5	-15.476	-12.574	-11.180	-14.255	-11.338	-10.014
5	Turkey toast	Boiled	A	0.977	6.4	11.2	-15.471	-12.617	-11.307	-14.274	-11.394	-10.069
6	Pariza - Salami	Boiled	B	0.971	6.6	25.7	-15.567	-12.815	-11.583	-14.370	-11.567	-10.336
7	Turkey fillet	Boiled	B	0.983	6.6	150.8	-15.848	-13.343	-12.352	-14.662	-12.098	-11.137

RISK CHARACTERIZATION-RISK PER SERVING

RISK CHARACTERIZATION-RISK PER SERVING

Ranking is highly related with nitrite concentration

RISK CHARACTERIZATION-RISK PER SERVING

Sensitivity Analysis

RISK CHARACTERIZATION-ANNUAL CASES

Consumption Data

Calculated annual eating occasions for each product category for consumers in Greece.

Product Category	*Consumers <65 yo*	*Consumers >65 yo*	*All Consumers*
Mortadella	1.48E+08	4.54E+07	1.93E+08
Parizer	3.93E+08	6.77E+07	4.64E+08
Boiled turkey	5.77E+08	7.98E+07	6.64E+08
Smoked turkey	7.72E+08	1.01E+08	8.84E+08
Boiled Ham	7.73E+07	1.33E+07	9.12E+07
Smoked Ham	9.34E+07	2.57E+07	1.19E+08
Smoked steak	3.89E+07	2.38E+06	4.21E+07
Boiled Chicken	3.62E+07	8.99E+06	4.52E+07
Smoked Chicken	5.09E+07	1.06E+07	6.17E+07
Total	**2.19E+09**	**3.55E+08**	**2.56E+09**

RISK CHARACTERIZATION-ANNUAL CASES

Results

Predicted annual listeriosis cases related with the consumption of RTE meat products sliced at retail in the Greek Market

	Consumers <65 yo			*Consumers >65 yo*			*All Consumers*		
	Median	95th	99th	Median	95th	99th.	Median	95th	99th
Parizer	0	0	0	0	1	2	0	1	2
Mortadella	0	0	1	0	1	1	0	1	2
Boiled turkey	0	0	0	0	1	1	0	1	1
Smoked turkey	0	1	1	0	1	1	0	2	2
Boiled Ham	0	0	0	0	1	1	0	1	1
Smoked Ham	0	0	0	0	0	1	0	0	1
Smoked steak	0	0	0	0	0	1	0	0	1
Boiled Chicken	0	0	0	0	1	1	0	1	1
Smoked Chicken	0	0	0	0	1	1	0	1	1
Total	**0**	**1**	**2**	**0**	**7**	**10**	**0**	**8**	**12**

SCENARIO ANALYSIS

1. Setting a use-by date in non-prepackaged ready-to-eat (RTE) cooked meat products

2. improving the temperature of domestic refrigerators

SCENARIO ANALYSIS

Results

UNCERTAINTY ANALYSIS

List of most important uncertainty sources and expected direction of their effect to model's outputs

Uncertainty Source	Description	Model Output	
		Risk per serving	Annual Cases
		Expected Direction of effect	
Concentration at the time of Slicing	Based on the fact that only 2 out of 300 samples were found positive the assumption that concentration of Lm follows a Poisson distribution is characterized by high uncertainty	+++	+++
Lag	Growth of Lm was considered without lag	-	-
Strain variability in the growth rate	The model used was based on experiments with a specific strain. Other strains may show different growth dynamics	-/+	-/+
Spoilage	Spoilage was not taken into account. However products stored at high temperature for long time may be spoiled before consumption	-	-
Sales of products	The sales of the different products within a product category were assumed to be same. However, a product with higher or lower risk may have different market share compared to other products	ne	-/+
Susceptible populations	Pregnant women were not taken into account due to absence of data	ne	++

+:expected to increase risk, -:expected to decrease risk, ne: no effect to risk expected

USING CONCENTRATION DATA FROM USA STUDY

USING CONCENTRATION DATA FROM USA STUDY

Objectives:

Objective 1 - Determine the occurrence of *Listeria monocytogenes* in 8015 selected RTE meat and poultry deli meats sliced at deli counter and product pre-packaged under USDA inspection

Objective 2 - Determine the levels of *Listeria monocytogenes (LM)* in RTE meat and poultry that is positive for LM

NAFSS

USING CONCENTRATION DATA FROM USA STUDY

LM Positive Deli Style RTE Meat & Poultry Samples -2005-2006

State	Pre-Packed	Deli Sliced	Total Pos	Total Samples
CA	0	12	12	2015
GA	0	10	10	2000
MN	4	12	16	2000
TN	2	15	17	2000
% POS	0.15%	1.23%	0.69%	8015
NFPA '03	0.4%	2.7%	0.89% (2000-2001)	

USING CONCENTRATION DATA FROM USA STUDY

USING CONCENTRATION DATA FROM USA STUDY

Results

	Consumers <65 yo			Consumers >65 yo			All Consumers		
	Median	95th Perc.	95th Perc.	Median	95th Perc.	99th Perc.	Median	95th Perc.	99th Perc.
Mortadella (Greece Poisson)	0	0	1	0	1	1	0	1	2
Mortadella (USA data)	1	3	4	2	4	5	3	7	9

SCENARIO ANALYSIS (USA DATA)

Results

CONCLUSIONS

- In total 87 products of non-prepackaged ready-to-eat (RTE) cooked meat products sliced at retail stores with 4 replicates for each product were analyzed for their physicochemical and microbial characteristics.
- Validation experiments showed a very good performance of the FSSP model in predicting the growth of L. monocytogenes in non-pre packed RTE meat products stored at both static and dynamic temperature condition.
- Analysis of data from 190 domestic refrigerators in Greece showed that the mean temperature ranged from -2.4 to 13.09 oC with an average value of 5.96 and a st. dev of 2.73 oC.
- A quantitative RA model predicting the risk of listeriosis related to the consumption of non-prepackaged ready-to-eat (RTE) cooked meat products handled at retail stores in Greece was developed. The model was used for ranking the 87 tested products based on the probability of illness per serving. In general the ranking was highly related with the concentration of nitrites. Products with low nitrite concentration showed a higher risk per serving and ranked higher.

CONCLUSIONS

- A sensitivity analysis showed that the initial prevalence and concentration of the pathogens immediately after slicing and the storage temperature and time at the domestic refrigerator had the higher impact on the probability of illness per serving.
- The risk assessment model was also applied for the product categories as defined in the consumer survey. In general the predicted number of cases was very low with the median value being zero for the total population. The predicted 95th percentiles of the listeriosis cases were 8 for the total population from which 1 case for consumers <65 years old and 7 cases for consumers >65 years old.
- Two scenarios for assessing potential interventions to reduce the risk of listeriosis in non-prepackaged ready-to-eat (RTE) cooked meat products were tested. 1)Setting a use-by date in non-prepackaged ready-to-eat (RTE) 2. Improving domestic storage temperature. Both scenarios resulted in the elimination of both the 95th and 99th percentiles of the number of annual cases.

CONCLUSIONS

- The most important uncertainty source for the model was the concentration of Lm at the time of slicing.
- The model was rerun for mortadella product using concentration data from a USA study. The results showed 3 times higher number of cases
- The two scenarios however resulted again to the elimination of the mean number of annual cases and to a significant decrease for the 95th and 99th percentiles

THANK YOU

QUESTIONS?

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