European Food Safety Authority

A European Union-wide slaughterhouse baseline survey on the prevalence of *Salmonella* in slaughter pigs

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Zoonoses Data Collection Unit

Outline

- Baseline Survey
- Material and methods
- Prevalence estimations
- Associated factor analysis
- Conclusions and Recommendations
- Acknowledgments

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- **Baseline Survey**
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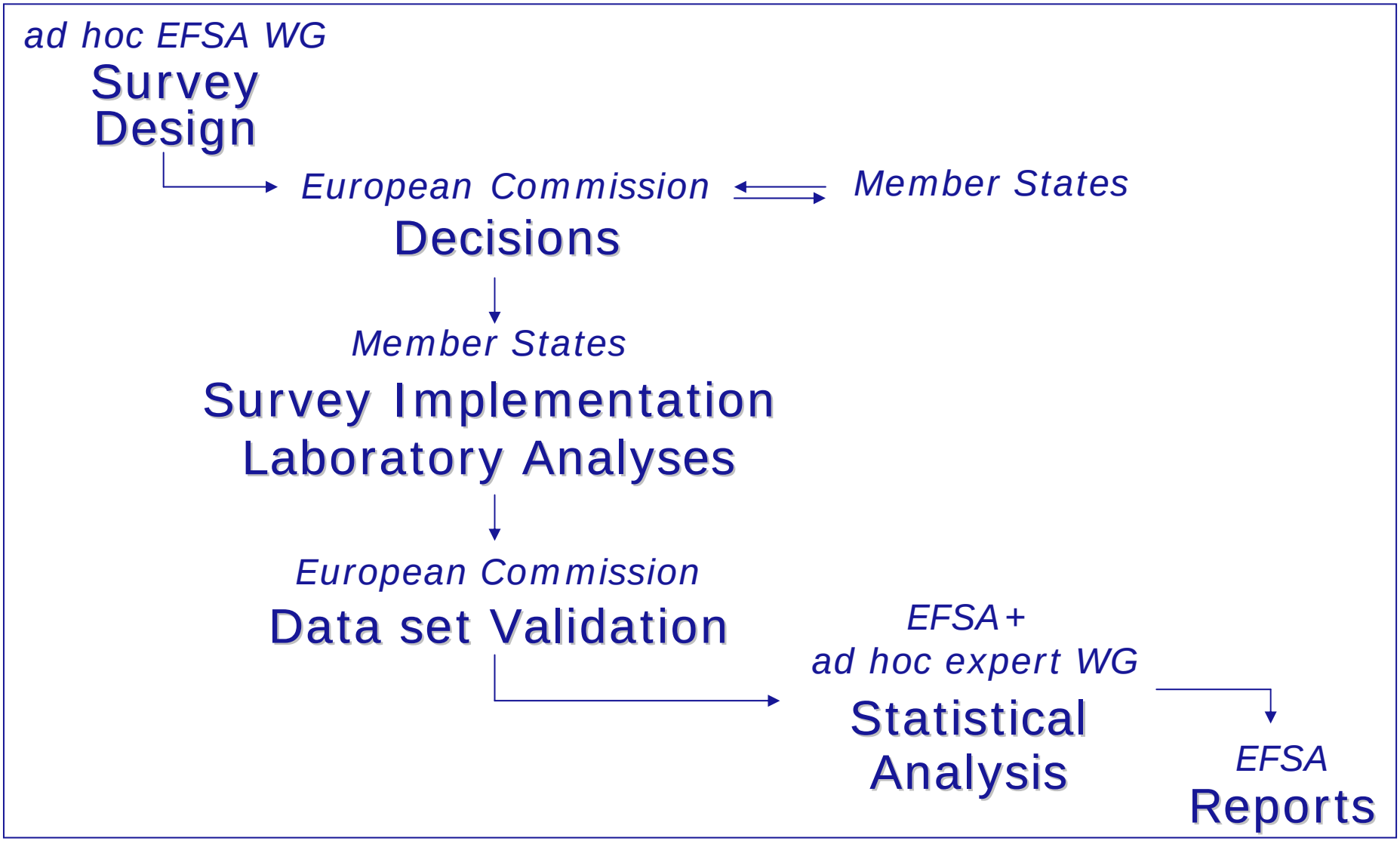
Baseline survey
Chart flow



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Material and methods (1)
Objectives and sampling procedure

- **Primary objectives**
  - Prevalence of slaughter pigs infected with *Salmonella* in lymph nodes
    - at the EU level
    - at the MS level
  - Prevalence of *Salmonella*–contaminated carcasses of slaughter pig
    - at the level of a group of voluntary MSs
    - for each MS individually
- **Sampling design**
  - **Units:** individual carcasses of slaughter pigs
  - **Sample size:** proportional to the live pig population (from 384 to 2,400 carcasses)
  - **Site:** slaughterhouses representing at least 80% of national throughput (2005)
  - **Sample:**
    - Stratified by slaughterhouses, and
    - Proportional to the slaughterhouse throughput (2005)
    - Random selection of sampling days and carcasses
    - Collected by the Competent Authorities from October 2006 to September 2007

Material and methods (2)
Samples from pigs

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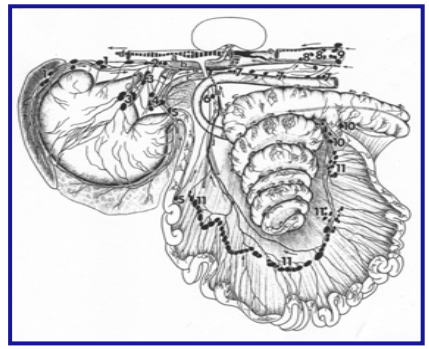
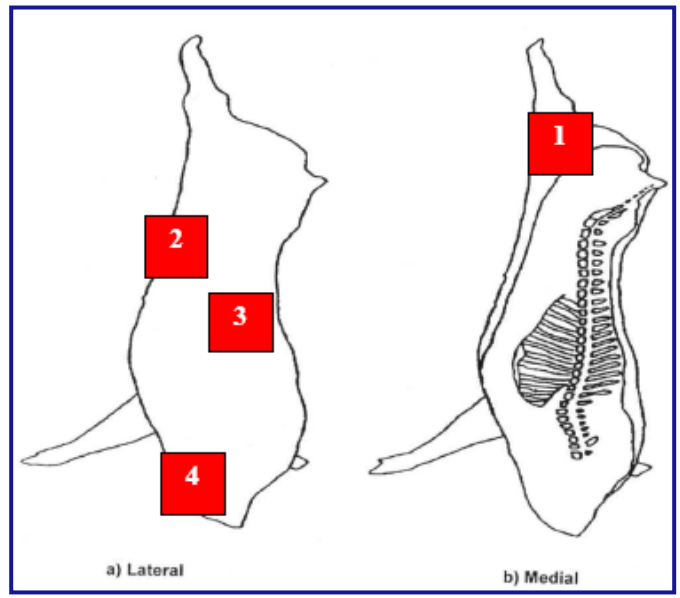
- Ileo-caecal lymph node samples
  - At least 5 lymph nodes **and** ≥ 15 gr. per carcass
  - 25 MSs + Norway



- Carcass swabs
  - After evisceration and before chilling
  - One sponge per carcass
  - Swabbed at four sites (100 cm² per site):
    - . Hind limb, medial 1
    - . Abdomen, lateral 2
    - . Mid-dorsal region 3
    - . Jowl 4
  - 13 MSs on a voluntarily basis
  - 384 pigs per MS were randomly sub-sampled from the included pigs



Material and methods (3)
Samples from pigs and questionnaire

- **Sense of the samples**
  - Lymph node
    - A sensitive test at the individual animal level
    - *Salmonella* infection of slaughter pigs at primary production
  - Carcass swabs
    - *Salmonella* surface contamination of the carcass
    - Contaminated carcass is likely to be a greater risk to public health as the carcass is the start of the food chain
- **Questionnaire**
  - Factors potentially associated with *Salmonella* positivity
  - Collected at the time of sampling by the competent authority

Material and methods (4)
Analyses and serotyping of samples

- **Analyses**
  - Samples sent to laboratories within 36 h. after sampling
  - Samples analysed within 24 hours following receipt, and
  - No later than 96 hours following the time of sampling
- ***Salmonella* National Reference Laboratories** (NRL)
- **Other laboratories**
  - involved in official controls and under supervision of the NRL
- **Normalised detection method**
  - Annexe D of **ISO 6579 Standard**
  - Pre-enrichment in **BPW**
  - Enrichment on **MSRV** medium plates
- **Serotyping**
  - According to the Kauffmann-White scheme

Material and methods (5)
Statistical analysis

- **Descriptive analysis**
  - "survey protocol" *vs*. "collected sample"
- **Estimation of prevalences**
  - GEE model
  - Standardised weights
    - clustering and disproportionate stratified sampling design
      - MS-level weight (reciprocal of the sampling proportion for throughputs)
      - SH-level weight: WY2 (reciprocal of the sampling proportion for pigs)
  - Observed prevalences
    - no correction made for imperfect Se and Sp
  - 4 outcomes were considered:

. *Salmonella* spp.
. *S*. Typhimurium
. *S*. Derby
. 'Other *Salmonella* serovars'

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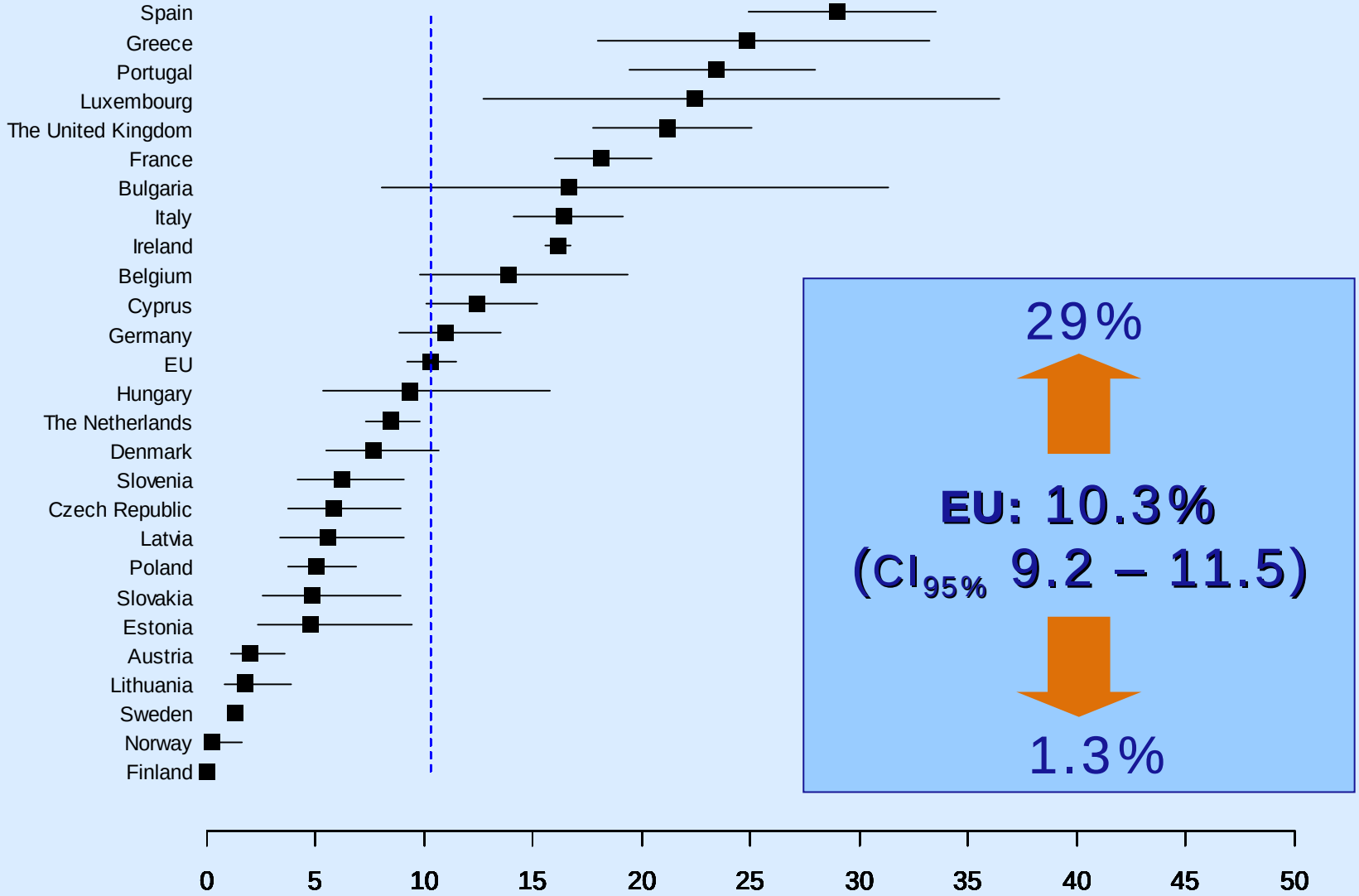
Results (1)
Data set

- **Data validation and cleaning**
  - 0.7% (141/19,300) of records were excluded from the dataset
- **Validated dataset**
  - 19,159 carcasses
    - → 19,071 lymph node samples
    - → 5,736 carcass swabs
  - 943 slaughterhouses

Results (2)
Slaughter pig infection by *Salmonella* spp.

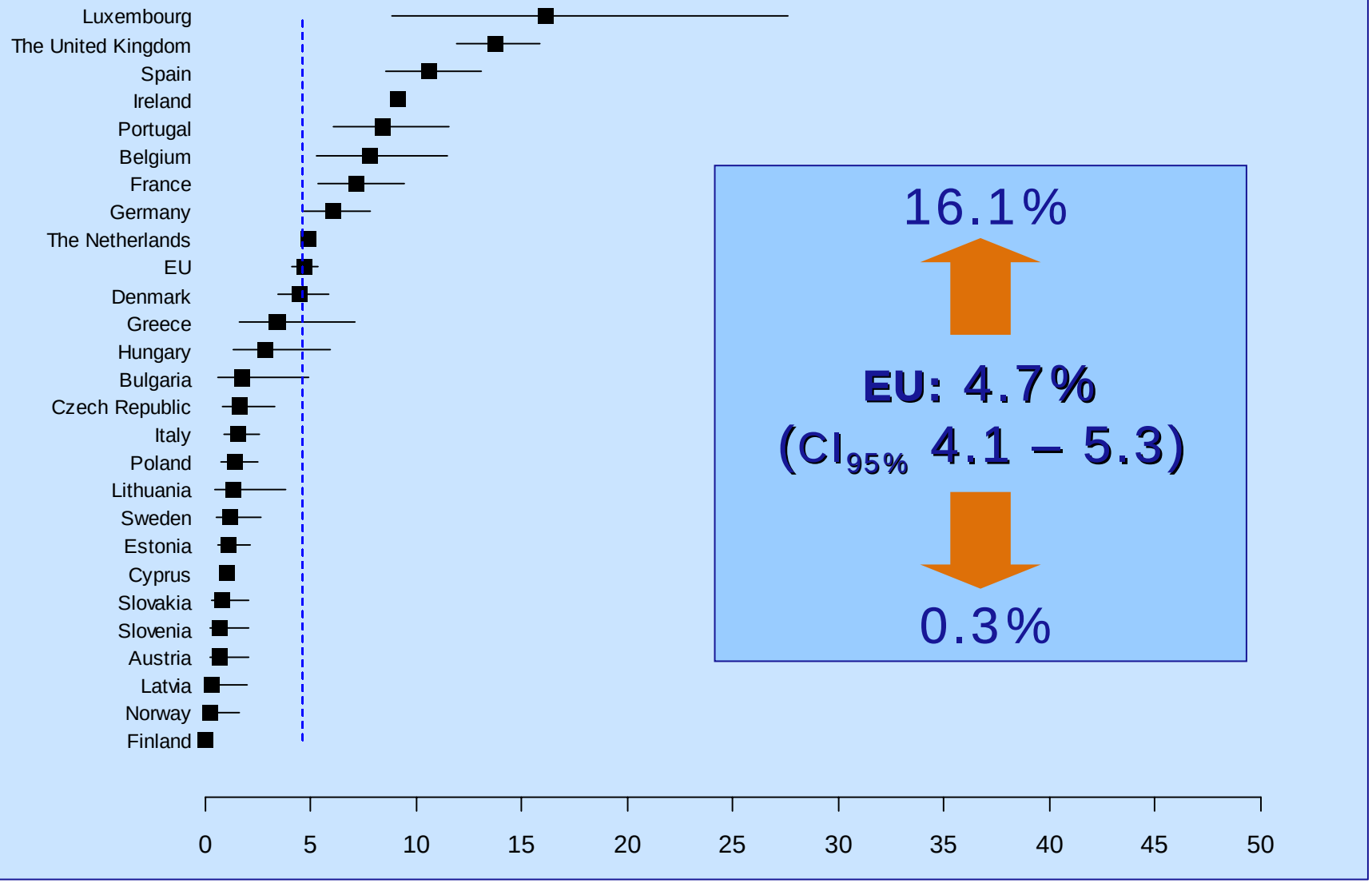


Results (3)
Salmonella serovars distribution

- Lymph nodes
  - **87 serovars** isolated from 2,600 positive lymph nodes (25 MSs + Norway)

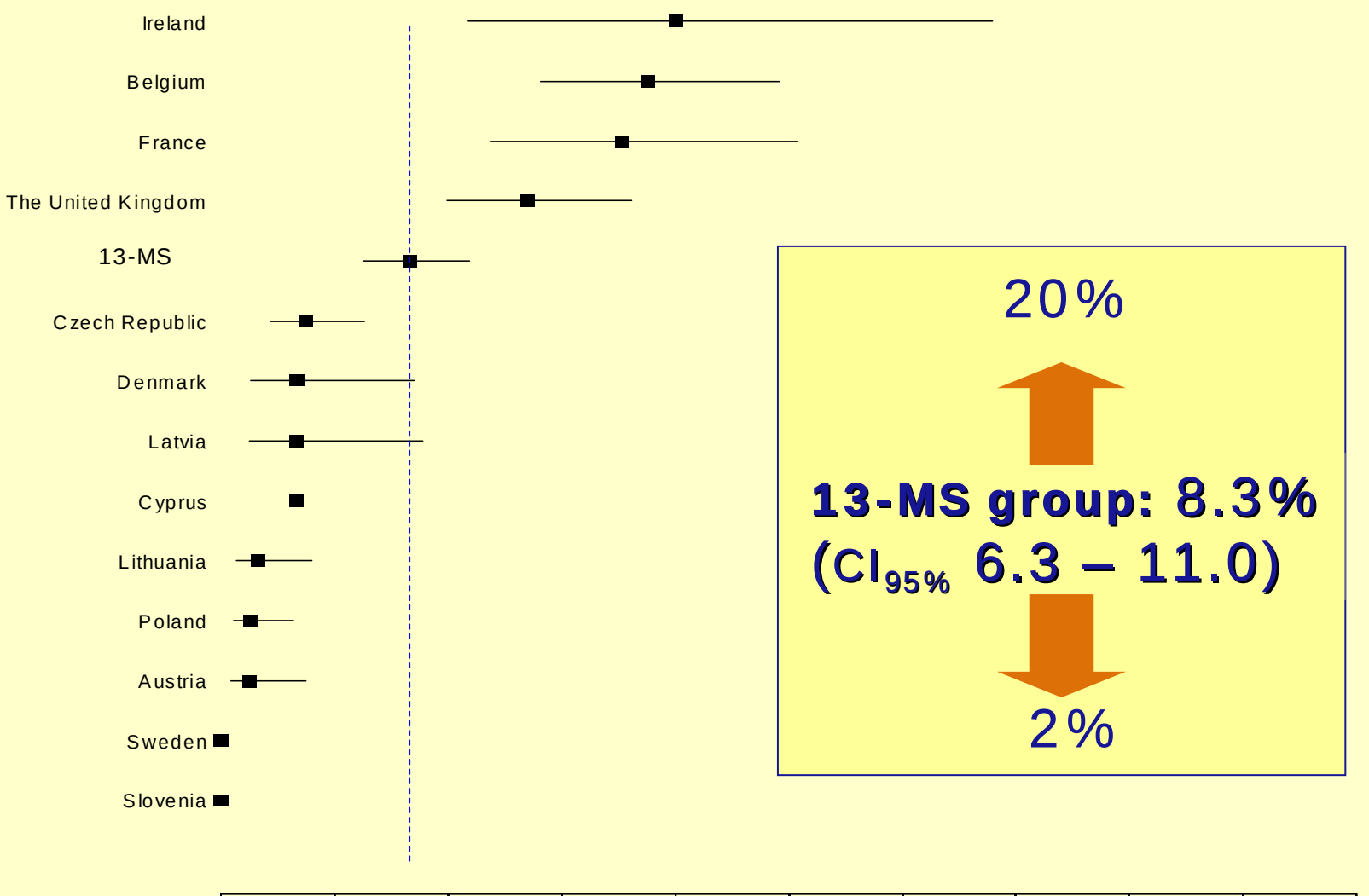
10 Most Frequent Serovars	N	%	Nb. of countries with serovars
S. Typhimurium	1,040	40.0	25
S. Derby	380	14.6	20
S. Rissen	151	5.8	5
S. 4,[5],12:i:-	128	4.9	8
S. Enteritidis	126	4.8	19
S. Anatum	63	2.4	10
S. Bredeney	51	2	9
S. Infantis	49	1.9	16
S. London	33	1.3	9
S. Brandenburg	31	1.2	7

Results (4)
Slaughter pig infection by ***S.* Typhimurium**



Results (5)

Carcass contamination by *Salmonella* spp.



20%

13-MS group: 8.3%

($CI_{95\%}$ 6.3 – 11.0)

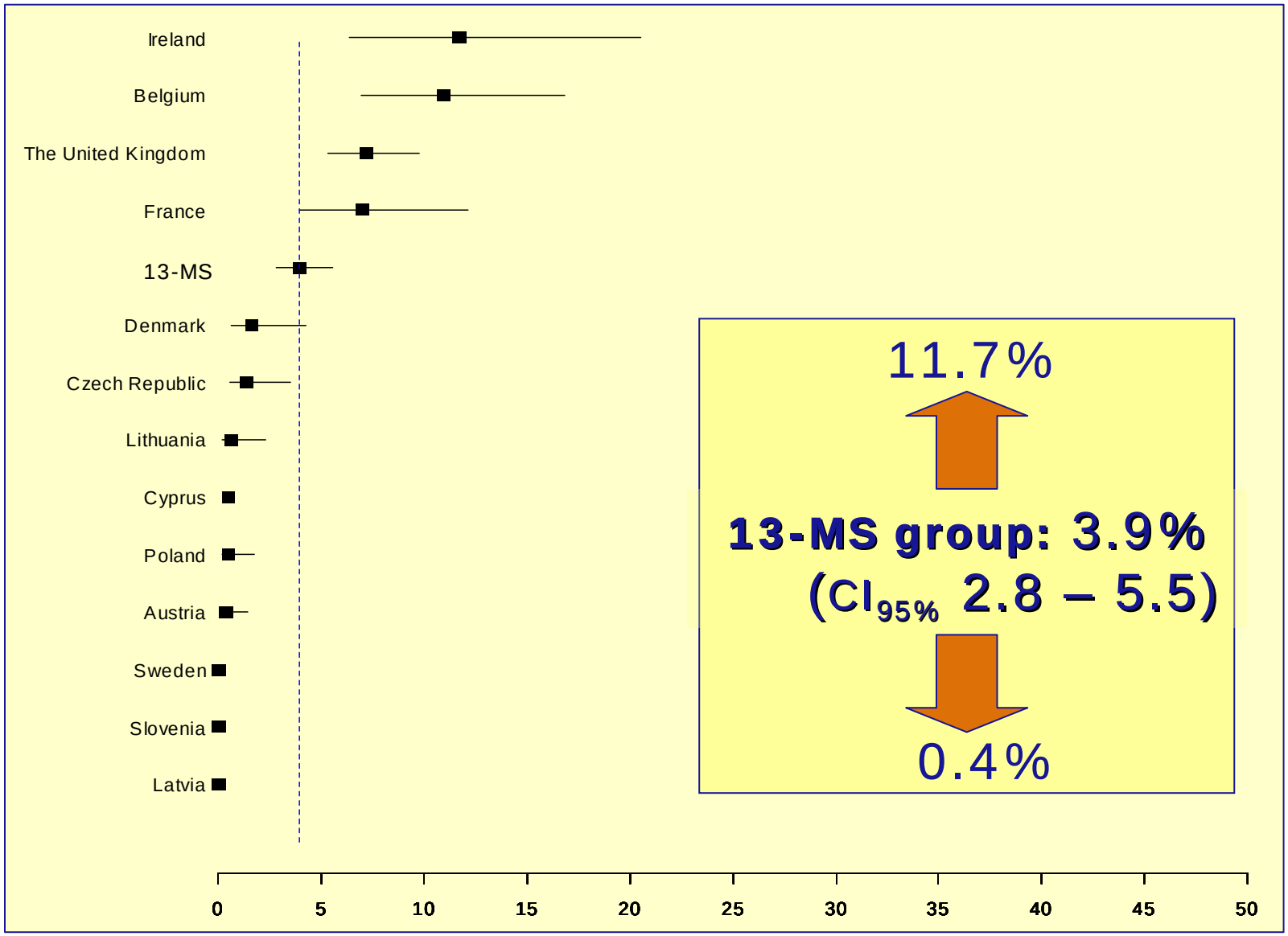
2%

Results (6)
Salmonella serovars distribution

- Carcass swabs
  - **30 serovars** isolated from 387 positive carcasses (13 MSs)

10 Most Frequent Serovars	N	%	Nb. of countries with serovars
S. Typhimurium	191	49	10
S. Derby	94	24	10
S. Infantis	13	3	5
S. Bredeney	8	2	4
S. Brandenburg	7	2	3
S. Reading	6	1.55	2
S. Enteritidis	5	1.29	3
S. Kedougou	5	1.29	2
S. 4,[5],12:i:-	5	1.29	1
S. Agona	4	1.03	3

Results (7)
Carcass contamination by *S.* Typhimurium



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Associated factor analysis
Objectives

- **To investigate the effect of potential factors on:**
  - *Salmonella* infection of slaughter pigs in the ileo-caecal lymph nodes
  - *Salmonella* surface contamination of slaughter pig carcasses

- **Factors:**
  - Sampling process sensitivity
  - Sample positivity: lymph node infection / carcass contamination

- To investigate the *Salmonella* serovar distribution in slaughter pigs across the EU } Report

Associated factor analysis
Salmonella in lymph nodes: Factors

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Factors tested

- **Factors related to the sampling process sensitivity**
  1. Weight of the lymph node sample
  2. Number of lymph nodes in the sample
  3. Time between sampling and testing (in days)

- **Factors related to lymph node infection**
  1. Month of sampling
  2. Hour of sampling in the slaughterhouse
  3. Weight of carcasses

Associated factor analysis
Statistical analysis

- **Preliminary steps**
  - Descriptive analysis of the factors
    - Graphical visualisations according to *Salmonella* status
  - Categorisation of variables
    - Time between sampling and testing: shape of distribution
    - Month of sampling → Quarter of sampling: seasonal effect
- **Random effect logistic model**
  - To account for slaughterhouse clusters
    - Random intercept for slaughterhouse
  - To deal with potential confounding between certain factors and countries
    - Fixed effect of the country

Associated factor analysis

Model of *Salmonella* infection of pigs

Variables	Random effect logistic model [a]	
	OR	95%CI
Weight of the lymph node samples [b]		
15-24gr	1	-
25-34gr	1.3	1.1 - 1.6
35-44gr	1.2	0.8 - 1.7
≥ 45gr	1.9	1.2 – 3.0
Time (in days) between the date of sampling and testing in the laboratory [b]		
0 to 2 days	1	-
3 to 4 days	1.2	1.04 – 1.4
5 to 7 days	0.99	0.65 – 1.5

- OR > 1.0 implies increased risk among pigs exposed
- OR < 1.0 implies a reduced risk among pigs exposed

Associated factor analysis
Main findings on pig infection

- Impact of the lymph node weight on the likelihood of detection of *Salmonella*

- The difficulties in standardisation of the lymph node sampling process should be considered when defining *Salmonella* control programme

Associated factor analysis
Salmonella on carcasses: Factors

Factors tested

- **Factors related to the sampling process sensitivity**
  1. Time between sampling and testing (in days)

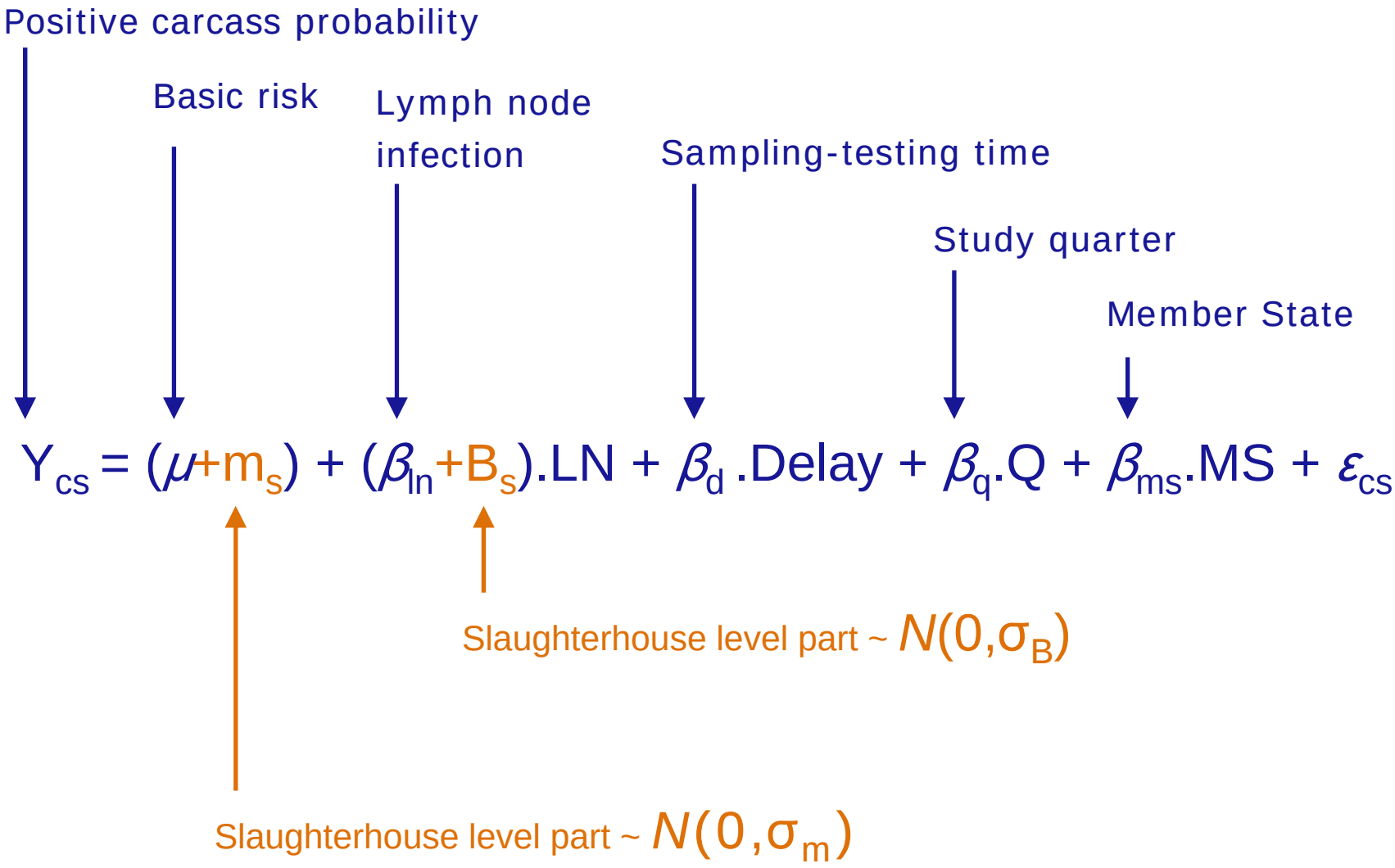
- **Factors related to carcass surface contamination**
  1. *Salmonella* status of live slaughter pig (lymph nodes)
  2. Month of sampling → Quarter of sampling
  3. Hour of sampling in the slaughterhouse
  4. Weight of the carcasses

Associated factor analysis
Salmonella on carcasses: Model

Variables	Random effect logistic model [a, b]	
	OR	95%CI
Time (in days) between the date of sampling and testing in the laboratory [c]		
0 day	0.51	0.28, 0.93
1 day	1	-
2 days	1.009	0.76, 1.3
3 to 7 days	0.70	0.52, 0.96
Lymph node infection of the live slaughter pig [c]		
No	1	-
Yes	1.8	1.1, 2.8
Quarter of sampling [c]		
Oct. – Dec. 2006	0.51	0.35, 0.72
Jan. – Mar. 2007	0.58	0.44, 0.77
Apr. – Jun. 2007	1.002	0.77, 1.3
Jul. – Sept. 2007	1	-

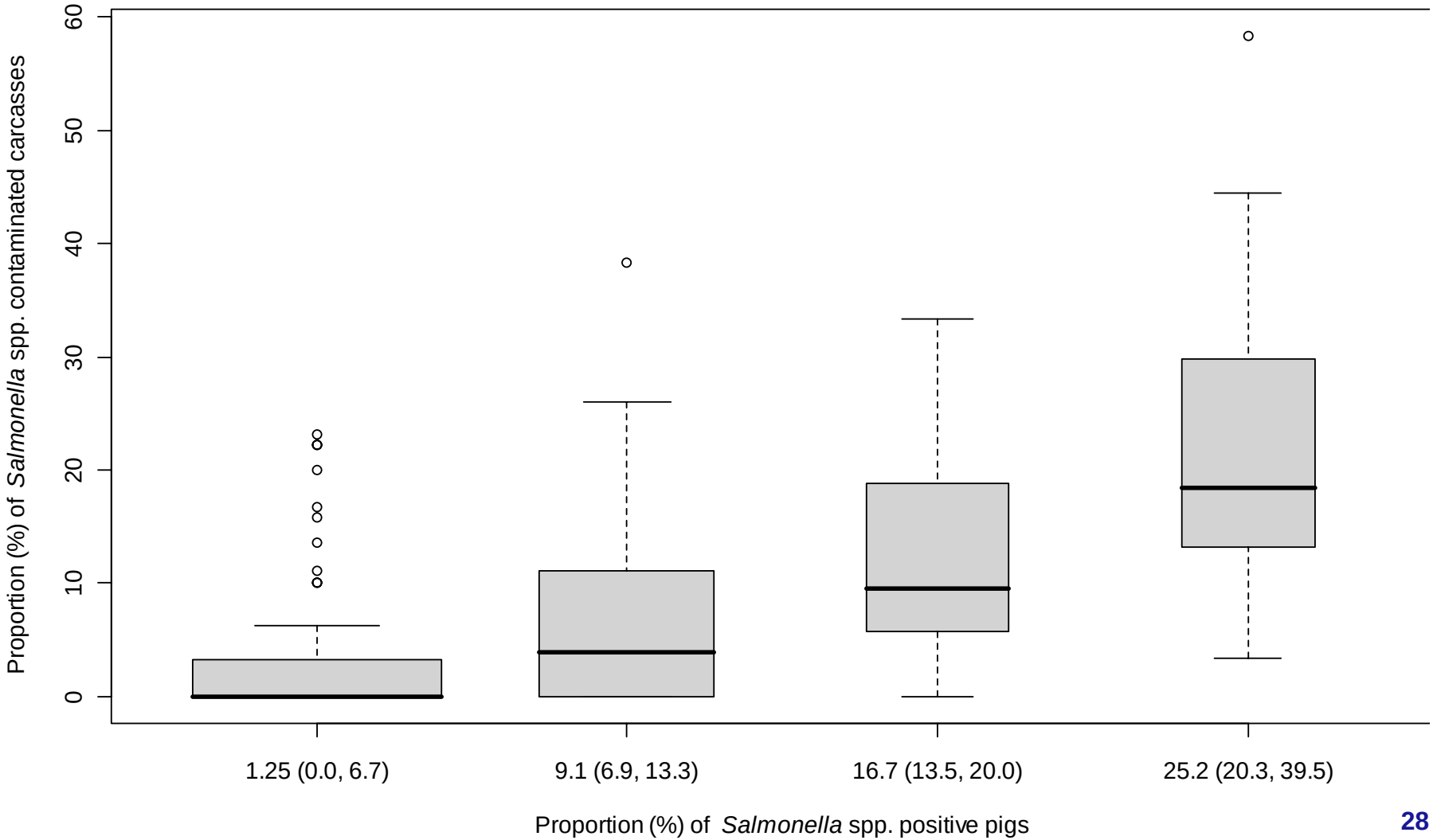
- Significant random slope of “LN infection” for the slaughterhouse

Associated factor analysis
Salmonella on carcasses: Model



Associated factor analysis

Salmonella on carcasses: “slaughterhouse effect”



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Associated factor analysis
Conclusions

- Association between *Salmonella* infection of slaughter pigs and carcass contamination
- The risk of carcass contamination varied significantly between slaughterhouses
- Certain slaughterhouses were more capable of controlling and preventing *Salmonella* contamination than others
- At EU level, the April-September 2007 survey period was more at risk → to be verified in individual MSs
- Sampling and testing procedure impacted on the likelihood of detection of *Salmonella*

Associated factor analysis
Recommendations

- Harmonisation of sampling and testing procedures should be considered of importance at the national and EU level
- Relevant studies on the survival rates of *Salmonella* in different relevant matrices

- Further analytical studies at the national level
- As pig infection and slaughterhouse process have both an impact on risk of carcass contamination:
  - Integrated control programme may prove to be a feasible and cost-effective option
  - MSs are encouraged to guarantee *Salmonella* controls at primary production as in the slaughterhouses
  - EU pig meat industry is invited to pay increased attention to slaughter hygiene and other relevant factors affecting *Salmonella* contamination of carcasses

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Acknowledgements

- **The competent authorities of MSs**
- ***Ad hoc* expert working group**

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- **More information available on EFSA web site**
  - *Part A Report* on prevalences

    http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178713190037.htm
  - *Part B Report* on associated factors analysis

    http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902236431.htm

Thank you for your attention!