

SCIENTIFIC REPORT OF EFSA

Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008¹

Part A: MRSA prevalence estimates

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major concern for public health. The MRSA lineage ST398 has been recognised as an occupational hazard for people in contact with pigs and it can occasionally be introduced into hospitals. This preliminary European Union-wide baseline survey on MRSA was conducted in 2008 in holdings with breeding pigs. A total of 1,421 holdings housing and selling mainly breeding pigs (breeding holdings), and 3,176 holdings housing breeding pigs and selling mainly pigs for fattening or slaughter (production holdings) from 24 European Union Member States were included in the survey. Also, two countries not belonging to the European Union participated in the survey. Pooled dust samples collected from the holdings were tested for MRSA and all isolates were typed by *spa*-typing and classified in relation to belonging to MRSA ST398. The survey results indicate that MRSA was commonly detected in holdings with breeding pigs in some Member States, while in other Member States prevalence was low. Seven Member States did not detect any MRSA in the surveyed holdings. The European Union prevalence of MRSA positive breeding holdings was 14.0%, and the prevalence varied from 0% to 46.0% among the Member States. The European Union prevalence of MRSA positive production holdings was 26.9%, while in Member States prevalence varied from 0% to 51.2%. MRSA ST398 was the predominant MRSA lineage identified, covering 92.5% of the MRSA isolates. MRSA isolates not belonging to ST398 were detected in six Member States. A varying set of MRSA *spa*-types was isolated, although the t011 *spa*-type was by far the most dominant type. MRSA *spa*-types not belonging to ST398 described in human medicine were also detected among the surveyed pig holdings. Monitoring of MRSA in food producing animal species as well as investigations of human health importance of the non-ST398 MRSA findings in pigs are recommended.

KEY WORDS

MRSA, *spa*-typing, MLST, pigs, breeding pigs, prevalence, EU.

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SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognised as an important cause of hospital-associated infections in humans for several decades. MRSA is resistant to the most commonly used antibiotics. The MRSA lineage ST398 (MRSA ST398) has been recently described as a cause of infection for people occupationally exposed to pigs, by direct or indirect contact. MRSA ST398 can occasionally be introduced into hospitals as a result of community-acquired human infections. To assess the occurrence and the diversity of MRSA in pig primary production, a European Union-wide preliminary survey was carried out in parallel with a baseline survey on *Salmonella* spp. in holdings with breeding pigs to determine the prevalence of holdings positive for MRSA and MRSA ST398.

Sampling took place between January 2008 and December 2008. Five dust samples were taken in the immediate environment of breeding pigs in the holdings. The pooled sample of each holding was tested for the presence of MRSA and all isolates were sub-typed by *spa*-typing and where necessary by Multi-Locus Sequence Typing (MLST). On the basis of typing results, isolates were classified as either belonging to MRSA ST398 or to other sequence types. A total of 1,421 holdings housing and selling mainly breeding pigs (breeding holdings), and 3,176 holdings housing breeding pigs and selling mainly pigs for fattening or slaughter (production holdings) from 24 European Union Member States were included in the survey. Also, two countries not belonging to the European Union (non-Member States) participated in the survey. Seventeen Member States detected MRSA in their breeding or production holdings whereas seven Member States did not detect any MRSA in the surveyed holdings. MRSA was more often detected in production holdings than in breeding holdings. The European Union prevalence of MRSA positive holdings with breeding pigs, as estimated based on the results from the 24 participating Member States, was 22.8%. MRSA ST398 was the predominant MRSA lineage identified in the holdings with breeding pigs in the European Union, counting for 92.5% of the MRSA isolates.

The results were analysed separately for breeding holdings and production holdings, due to the expected differences in MRSA occurrence in those types of holdings. Twelve of the 24 Member States isolated MRSA in breeding holdings, which resulted in a European Union prevalence of breeding holdings positive for MRSA and for MRSA ST398 of 14.0% and 13.1%, respectively. Prevalence varied widely among the Member States, from 0% to 46.0%. One Member State reported MRSA isolates not belonging to lineage ST398 (MRSA non-ST398). Neither of the two non-Member States found MRSA in their breeding holdings.

Sixteen Member States and one non-Member State detected MRSA in production holdings, while eight Member States did not. The European Union prevalence of MRSA and of MRSA ST398 positive production holdings was 26.9% and 25.5%, respectively. The prevalence of MRSA and of MRSA ST398 positive production holdings also varied widely among MSs, from 0% to 51.2% and from 0% to 50.2%, respectively. Only six Member States and one non-Member State reported isolates of MRSA non-ST398, resulting in a low European Union prevalence of non-ST398 positive production holdings of 1.4% (range from 0% to 12.3%).

The prevalence presented in the report are observed prevalence, meaning that the prevalence estimates do not account for potential imperfect test characteristics. The MRSA prevalence estimates from this preliminary survey may underestimate the true prevalence due to a possible lack of sensitivity of the pooled environmental samples taken.

In this survey, the most frequently isolated *spa*-types belonging to MRSA ST398 were t011, t108 and t034. In addition, *spa*-types belonging to MRSA non-ST398 were detected in production holdings and, to a lesser extent, in breeding holdings. In particular, *spa*-types belonging to lineages ST5, ST8, and ST132, which are *spa*-types known in human medicine, were each isolated in different production holdings. Colonisation of pigs with MRSA ST398 has been identified as an occupational health risk for farmers, veterinarians and their families. Such information is not available for the MRSA non-ST398 strains isolated in this survey from pig holdings and which have also been detected in humans.

It is recommended that the information from this preliminary survey be complemented by monitoring of MRSA in breeding and fattening pigs as well as in other food-producing animal species such as poultry and cattle. Also investigations of the causes of variation in MRSA prevalence among the Member States as well as of the human health importance of the MRSA non-ST398 findings in pigs and the role of humans as potential sources of these strains are recommended.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is known for its impact on public health. MRSA is typically a health care related problem (hospital-acquired MRSA), although increasingly community-acquired strains (CA-MRSA) are also reported. Recently, the MRSA, clonal complex ST398 seems to have found a reservoir in animals and it has been reported to occur in pigs, cattle, horses, poultry and dogs. This MRSA clone has been shown to be capable of infecting humans and therefore this clone of MRSA is a zoonosis with a direct public health impact. For certain professional groups (e.g. pig holders and their families, veterinarians) it might be considered as an occupational health risk.

Triggered by the Dutch findings on the presence of MRSA in pigs and other food-producing animals and the spread to pig holders, an increasing number of studies from other countries demonstrate the presence of MRSA in several animal species, including pet, wild and farm animals. This includes a report on a prevalence survey of MRSA in Belgium in pigs, in pig farmers and in other human populations. MRSA has been found in both breeding and finishing pig herds in the Netherlands.

Upon a request of the European Commission, the European Food Safety Authority (EFSA) adopted a “Report of the Task Force on Zoonoses Data Collection on a proposal for technical specifications for a baseline survey on the prevalence of MRSA in breeding pigs”.

Based on the EFSA proposal, the Commission adopted Decision 2008/55/EC of 20 December 2007 (EC, 2007b) concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. and Methicillin-resistant *Staphylococcus aureus* in herds of breeding pigs to be carried out in the Member States (MSs). The survey started on 1 January 2008 for a period of 12 months. The survey should provide:

- a comparison of the prevalence of MRSA in herds of breeding pigs; and
- information on risk factors contributing to the prevalence.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is asked to analyse the results of the baseline survey as regards Methicillin-resistant *Staphylococcus aureus* (MRSA), in particular:

- to estimate the prevalence of MRSA in herds of breeding pigs in MSs and at EU level; and
- to evaluate the risk factors for MRSA in herds of breeding pigs based on the information collected.

ANALYSIS

1. Introduction

This report (part A) describes the results of a preliminary baseline survey carried out in the EU to estimate the prevalence of MRSA in holdings with breeding pigs. This survey was performed in parallel with the baseline survey on *Salmonella* spp. in holdings of breeding pigs during the same visits to selected holdings. The objective of the survey has been to obtain preliminary comparable data on the occurrence of MRSA for all MSs through a harmonised sampling scheme. The report part B on the analyses of the baseline survey on MRSA in holdings of breeding pigs will describe the results of the analyses of factors associated with the occurrence of MRSA in pig holdings and will be published at a later date.

MRSA, which is resistant to the most commonly used antibiotics, has been recognised as an important cause of infection in hospitals for several decades; strains of MRSA have also emerged which are particularly associated with community-acquired infections in humans. An apparently new development has been the recent detection of MRSA lineage multi-locus sequence type 398 (ST398) in production animals in several MSs. In particular, pigs have been acknowledged as an important source of colonisation of this MRSA strain for pig farmers, veterinarians and their families, through direct or indirect contact with pigs. MRSA ST398 has therefore been considered an occupational hazard for humans. This newly recognised strain, which appears to be primarily acquired by occupational exposure, can on occasion be introduced into hospitals. More background information on MRSA can be found in EFSA's assessment of the public health significance of MRSA in animals and food (EFSA, 2009). In order to increase awareness and to assess the occurrence of MRSA in pig primary production across the EU, comparable data on the occurrence of MRSA and MRSA ST398 in pig holdings in MSs are needed.

Such comparable information was not available and therefore a special survey was carried out to estimate the prevalence of MRSA positive pig holdings, in accordance with Article 5 of Directive 2003/99/EC (EC, 2003) on the monitoring of zoonoses and zoonotic agents. The Directive notably aims to establish coordinated monitoring programmes, especially when specific needs are identified, to assess risks at MS or at Community level. Taking into account the public health significance of MRSA, the emerging risk of pigs as a source of infection for humans, and the lack of comparable information on the prevalence of MRSA in pig holdings across Europe, it was acknowledged to be most cost-effective to conduct an preliminary survey evaluating the prevalence of MRSA in holdings with breeding pigs in the Community concomitantly with the survey provided for in Decision 2007/636/EC (EC, 2007a) on the prevalence of *Salmonella* spp. in herds of breeding pigs, which was already planned and close to its launch.

The survey was carried out over a one-year period, between 1 January 2008 and 31 December 2008 in holdings with breeding pigs. The target population were holdings harbouring at least 80% of the breeding pig population per MS and included in most MSs two sub-target populations: breeding holdings and production holdings with breeding pigs. Breeding holdings sell a proportion of gilts or boar for breeding purposes, while the remainder is sold for slaughter. Production holdings mainly sell growing pigs for fattening or provide slaughter pigs directly to the slaughterhouse. Production holdings with breeding pigs may be of farrow-to-weaner, farrow-to-grower or farrow-to-finish types. Thus, the remaining types of production holdings, namely the weaner-to-finish and finisher holdings, were not targeted by this survey. Figure 1 shows the pyramidal structure of the pig primary production sector and shows the breeding and production holding types included in the survey. Detailed definitions of the different types of pig holdings are given in the glossary.

The two types of holdings housing breeding pigs (breeding holdings and production holdings) are usually distinguished. They are likely to differ in terms of management and hygiene practices, pig-

health status, and biosecurity measures in place. Breeding holdings are generally considered to have better status with regard to these aspects. In addition, breeding holdings provide breeding pigs to production holdings and may thus vertically disseminate MRSA. Therefore, separate investigations of the situation in breeding holdings and production holdings are meaningful. Hereafter in this report, these two types of holdings with breeding pigs will be referred to as ‘breeding holdings’ and ‘production holdings’ for brevity.

Twenty-four EU MSs participated in the survey whereas Greece, Malta and Romania did not carry out the survey. In addition, two countries not belonging to the EU, Norway and Switzerland (subsequently referred to as non-MSs) participated in the survey.

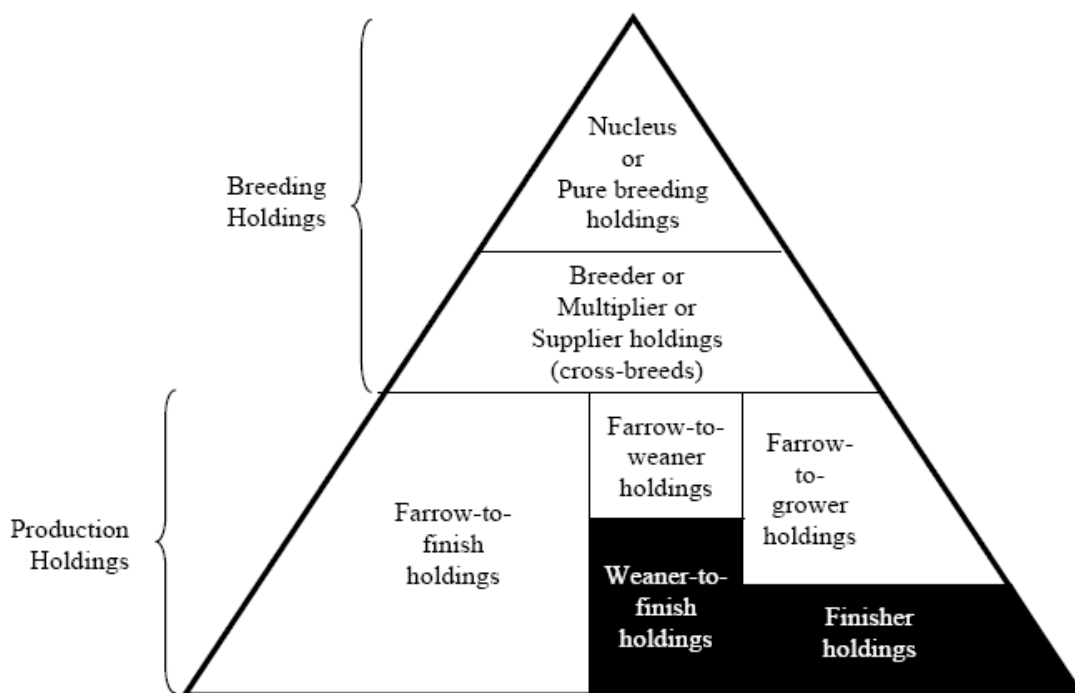


Figure 1: Overview of the pig breeding and production holdings included in the EU MRSA baseline survey in breeding pigs, 2008. Weaner-to-finish and finisher holdings are not covered by the survey⁴

2. Objectives

The aim of the survey was to estimate the prevalence of MRSA positive breeding pig holdings and of MRSA positive production holdings with breeding pigs, separately, at Community level, as well as for each MS.

The specific objectives for breeding holdings and for production holdings with breeding pigs were:

- to estimate the prevalence of MRSA positive holdings at EU level and for each MS individually;
- to estimate the prevalence of MRSA ST398 positive holdings at EU level and for each MS individually;

⁴ Please refer to Glossary at end of report for definitions.

- to estimate the prevalence of MRSA non-ST398 positive holdings at EU level and for each MS individually;
- to investigate the *spa*-type distribution and determine the most frequently occurring *spa*-types in pig holdings with breeding pigs across the EU; and
- to investigate the effect of potential factors, such as the number of pigs per holding and time of sampling, which may be associated with the occurrence of MRSA.

MSs were also invited to submit additional information on the antimicrobial susceptibility of MRSA isolates, but this testing was not a compulsory requirement of the survey.

This part A report describes the results of the analyses of the prevalence of MRSA and of the most frequent *spa*-types. The analysis of the factors potentially associated with MRSA prevalence, the analysis of the association between breeding and production holding prevalence, as well as the more in-depth analyses of MSRA *spa*-types and clonal complex distribution will be reported in the part B report.

3. Materials and methods

A detailed description of the design of the baseline survey, sampling scheme, sample size, bacteriological testing, *spa*-typing and the Multi-Locus Sequence Typing (MLST) is found in Annex I of Commission Decision 2008/55/EC of 20 December 2007 concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. and Methicillin-resistant *Staphylococcus aureus* in herds of breeding pigs to be carried out in MSs (EC, 2007b).

3.1. Survey design

Environmental dust samples were taken from the immediate environment of breeding pigs in breeding holdings and in production holdings (i.e. farrow-to-weaner, farrow-to-grower, and farrow-to-finish holdings). The survey was carried out on holdings harbouring at least 80% of the breeding pig population in a MS, which was to be achieved by including, preferentially, holdings with 50 breeding pigs or more. A breeding pig means a pig (sow or boar) of at least six months of age kept for breeding purposes. Whenever the selected holdings with at least 50 breeding pigs did not contain 80% of the national herd of breeding pigs, smaller holdings with less than 50 breeding pigs were also sampled. A detailed description of the 2008 populations of breeding and production holdings in the EU is given, as reported by the participating countries, in Appendix A. The enrolled breeding holdings and production holdings included in the survey were randomly selected.

In each selected breeding or production holding, five dust samples were collected using five dry sterile swabs of about 500 cm² each from five pens chosen such that pens with breeding pigs over six months of age were included. These were proportionally allocated in different production stages (e.g. service period, pregnancy phase, suckling phase) and other categories of breeding pigs (boar, etc.), except those in quarantine. Every set of five samples from one holding was pooled in the laboratory for analysis (see section 3.2.). Samples were taken by the competent authority in each MS or under its supervision.

3.2. Isolation, identification and typing of MRSA

The environmental dust samples were tested for the presence of MRSA and isolates sub-typed by the National Reference Laboratories (NRLs) for *Staphylococcus aureus* and/or antimicrobial resistance, or an authorised experienced laboratory. In the laboratory the five dust swabs were pooled in Mueller-Hinton Broth with 6.5% NaCl and incubated for 16-20 hours at 37°C. Following pre-enrichment, selective enrichment was performed in Tryptone Soy Broth (TSB) containing

cefoxitin and aztreonam and incubated for a further 16-20 hours at 37°C. Plating included streak inoculations from TSB onto chromogenic MRSA-selective agar plates, incubated for 24 to 48 hours at 37°C. Up to five presumptive positive colonies on a MRSA selective medium were sub-cultivated on blood agar for 24 to 48 hours at 37°C. One presumptive positive colony on blood agar was subjected to confirmatory testing for *S. aureus* and MRSA using either a multiplex Polymerase Chain Reaction (PCR) with simultaneous identification of the *mecA*-gene and an *S. aureus* species-specific gene or using two different, separate PCRs to achieve the same end. If the first isolate of the initial five isolates was not identified as MRSA, the next isolate was tested until one MRSA had been identified or all five isolates had been tested. Alternatively, identification by PCR as a first step could be done on a pool of the five presumptive colonies from a sample. In case of a positive PCR, the analysis had to be repeated on individual colonies to identify a positive colony. MRSA isolation was to be started within 13 days of sampling.

All isolates were initially tested by *spa*-typing (*Staphylococcus* protein A typing). Typing was performed at the NRL or under its supervision, or isolates were forwarded to the Community Reference Laboratory for antimicrobial resistance (CRL-AR) to perform the typing. In general, isolates with a similar succession of *spa* sequences belong to closely related sequence types (ST), which can be assigned to the same clonal complex (CC). The knowledge of the *spa*-type generally enabled evaluation of whether the MRSA isolate belonged to the ST398/CC398 lineage. Detailed definitions of *spa*-typing, ST and CC are given in the glossary.

For quality assurance, a selection of presumptive *S. aureus* isolates which were not identified as MRSA, as well as 16 MRSA strains, from each MS were to be sent to the CRL-AR. Antimicrobial susceptibility testing of MRSA isolates was optional.

3.3. Data collection and validation

A set of data exclusion criteria (Appendix B) was used by the European Commission (EC) to identify and exclude non-valid and non-plausible information in the MRSA dataset submitted by MSs. MSs corrected the excluded data. Nevertheless, a marginal number of holdings was finally excluded. The reasons for excluding holdings, in accordance with exclusion criteria, could not be exhaustively addressed because relevant information was not fully available in some case.

This resulted in a cleaned, validated dataset comprising 5,073 holdings with breeding pigs in 24 MSs, and in two non-MSs (final dataset), which formed the basis for all subsequent analyses. No data was submitted by Greece, Malta or Romania. EFSA received the validated dataset from the EC on 24 June 2009. An overview of the validated dataset at holding level is given in Table 1.

3.4. Multi-locus sequence type and clonal complex assignments

A convenient sub-set of isolates belonging to different *spa*-types (about 2% of the number of pooled samples) was also subjected to MLST-typing (see glossary) performed by either the NRL or the CRL-AR. MLST-typing gives the ST of a given isolate, which can be allocated to given specific CCs. At least one isolate belonging to each new *spa*-type identified during the survey, as well as representatives of the major groups of *Spa*-types found, were MLST-typed to confirm concordance between *spa*-typing and allocation of a given isolate to a specific ST/CC. The STs identified were reported by MSs or CRL-AR to EFSA.

For the remaining non-MLST typed isolates, direct assessment of the ST was carried out by comparing their *spa*-types with databases of known and typed strains. Information derived from databases was also used to assign isolates directly to known CCs. All MRSA isolates were subsequently categorised into two groups: ST398 and non-ST398.

3.5. Phylogenetic analysis of the relationship between the *spa*-types isolated

To examine further the variation in *spa*-types identified in this baseline survey, all *spa*-types submitted by the MSs and non-MSs to the CRL-AR were inserted into the software programme Bionumerics v4.6 and analysed by the minimal Spanning tree algorithm. This created phylogeny trees based on *spa*-repeats which served as an initial grouping of the isolates within certain related *spa*-groups. The result of the MLST analysis described above was compared to these *spa*-groups in order to confirm the correct distribution into specific clonal complexes.

Table 1: Overview of the validated data set at holding level, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	Final validated dataset		
	Total number of holdings	Number of breeding holdings	Number of production holdings
Austria	242	75	167
Belgium	199	15	184
Bulgaria	72	47	25
Cyprus	69	4	65
Czech Republic	267	106	161
Denmark	293	95	198
Estonia	34	6	28
Finland	198	48	150
France	342	157	185
Germany	201	46	155
Hungary	181	40	141
Ireland	189	40	149
Italy	214	43	171
Latvia	33	5	28
Lithuania	82	10	72
Luxembourg	44	3	41
Netherlands	321	109	212
Poland	321	143	178
Portugal	170	34	136
Slovakia	192	96	96
Slovenia	114	27	87
Spain	359	150	209
Sweden	202	55	147
United Kingdom	258	67	191
European Union	4,597	1,421	3,176
Norway	251	108	143
Switzerland	225	71	154
Total	5,073	1,600	3,473

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

3.6. Statistical analysis

3.6.1. Descriptive analysis

A comparison between the survey protocol and the collected sample in terms of sample size, stratification by month, and time elapsed between sampling and testing, was carried out using frequency tables and graphs.

3.6.2. Estimate of the observed prevalence of MRSA positive holdings

Data on breeding holdings and production holdings were analysed separately, and the following three outcomes were considered for both types of holdings: positivity for MRSA (all types), positivity for MRSA ST398, and positivity for MRSA non-ST398. A holding was considered positive if either MRSA, MRSA ST398 or MRSA non-ST398 was detected respectively in the pooled dust sample, and negative otherwise. The prevalence of infection with MRSA ST398 was estimated separately as this lineage of MRSA may be of particular public health interest.

Prevalence was estimated for each MS through the breeding/production holding positivity ratio (proportion of test positive holdings out of the total number of holdings tested). All the data, including those from holdings with less than 50 breeding pigs, are included in the estimation of MS level prevalence.

At EU level, the prevalence was estimated using only the data from pig holdings with at least 50 breeding pigs. This approach was taken because the survey targeted holdings with at least 50 breeding pigs. Holdings with less than 50 breeding pigs were only to be sampled in those MSs that did not have a sufficient number of pig holdings to cover the sample size needed, which was only the case in a few MSs. At EU level, the proportion of sampled holdings with less than 50 breeding pigs was small (3% and 5% of the breeding and production holdings sampled, respectively). In addition, data on the total number of small holdings was not available from some MSs. Furthermore, including small holdings in the estimation of the EU prevalence, would have meant extrapolating findings chiefly derived from big holdings to a large number of small holdings in the EU, while a very limited number of those small holdings were sampled. Making such an extrapolation would assume that the risk of MRSA colonisation is the same in small and big holdings, and there is no information currently available to justify that assumption.

In the estimation of EU prevalence, MSs were considered as strata, and the proportion of sampled breeding/production holdings was not constant across MSs. To account for unequal sampling fractions among MSs, i.e. disproportionate sampling among MSs, the EU level prevalence was estimated as the weighted mean of the MS prevalence (see formula (1) in Appendix C), weighting each sampled holding with the reciprocal of the sampling fraction for breeding/production holdings (the total number of breeding/production holdings with at least 50 breeding pigs in a MS divided by the number of sampled breeding/production holdings with at least 50 breeding pigs in the same MS) (see formula (2) in Appendix C). In case the number of holdings in the sample was larger than the number of holdings reported by the country, the sampling fraction was assumed to be one (i.e. 100%).

This report presents estimates for MS level and EU level observed prevalence, meaning that the prevalence estimates do not account for test misclassification bias, i.e. imperfect sensitivity or specificity of the test. A finite population correction was used to calculate a 95% Confidence Interval (CI) of prevalence estimates at MS and EU level. More details on statistical approach and weighting are given in Appendix C.

Factors, such as the delay between the sampling date and testing date at the laboratory and the month of sampling, were considered to be potentially related to the sensitivity of the testing scheme and may have a potential impact on the probability of detection of MRSA isolates in samples. To investigate any such potential impact, both logistic models predicting holding positivity as a function of country and respectively, and the delay in testing after sampling and the month of sampling, were fitted separately for breeding holdings and production holdings. In case of impact, further regression analyses are warranted to investigate the estimation of adjustment prevalence figures.

4. Results

4.1. Overview of the 2008 populations of breeding and production holdings in the EU

An overview of the populations of breeding holdings and production holdings in the EU, stratified by the number of breeding pigs (more or less than 50 breeding pigs per holding), is presented, as reported by MSs, and non-MSs in Appendix A. The EU population of breeding holdings with more than 50 breeding pigs totalled 5,422 units in 2008. Poland had the highest number of such breeding holdings and accounted for 29.6% of the EU population. Conversely, several MSs, as well as the non-MSs, had a very low number of breeding pig holdings. The EU population of production holdings with more than 50 breeding pigs totalled 54,157 holdings in 2008. The largest populations were in Spain and Germany, representing 45% of those pig holdings. Conversely, several MSs had a very low number of such production holdings. These MS-specific figures of 'total numbers of breeding/production holdings with at least 50 breeding pigs' impact on the estimation of the EU prevalence as explained in section 3.6.2., because they determine – together with the sampled number of breeding/production holdings – the weight attributed to each sampled holding.

4.2. Sample summary statistics and protocol-sample comparison

The cleaned validated dataset (Table 1) comprised data on 1,421 breeding holdings and 3,176 production holdings with breeding pigs originating from 24 MSs, as well as 108 and 71 breeding holdings, and 143 and 154 production holdings from Norway and Switzerland, respectively. The number of breeding holdings included in the survey at MS level varied from three in Luxembourg up to 157 in France, whereas the number of production holdings investigated ranged between 25 in Bulgaria and 212 in Netherlands. The dataset also included data, from the two non-MSs on 108 and 71 breeding holdings, as well as on 143 and 154 production holdings, respectively. The sampling fractions and the sampled holding weights (detailed for breeding holdings and production holdings, with at least 50 breeding pigs), used to compute the EU level prevalence, are presented in Appendix D. For production holdings, sampling fractions range from one in Estonia and Luxembourg (all production holdings with at least 50 breeding pigs sampled) to 0.017 in Spain and 0.013 in Germany (meaning that 2% and 1% of the production holdings with at least 50 breeding pigs were respectively sampled in those countries, characterised by the important primary pig production sector). This results in heavy weights given to the positive sampled holdings of those last two countries when calculating EU level (weighted) prevalences.

The results of the descriptive analysis are presented in Appendix E. A summary of these results is presented thereafter.

The distribution of the number of holdings included in the survey by the month of sampling was represented for breeding holdings and production holdings. Sampling appears, in general, to be evenly distributed over the year by most participating countries, although greater numbers of production holdings were sampled during the last three months of the survey compared to the first ones. Portugal performed the entire survey during the last two months of the survey period.

The distribution of the holding size (number of breeding pigs) is presented for breeding holdings and production holdings. Overall, more than 60% of the breeding holdings and production holdings with breeding pigs sampled, housed between 100 and 999 breeding pigs.

The distribution of the number of dust samples by the number of days delay between sampling and testing for MRSA is displayed for the breeding and production holdings sampled. Both distributions are bimodal: two peaks occur, at one day and at seven days after sampling.

In addition, in Appendix F, the number and the proportions (%) of positive holdings, meaning the number of positive holdings out of the total number of sampled holdings, for each of the outcomes (all MRSA/MRSA ST398/MRSA non-ST398) are also displayed in breeding holdings and production holdings at both MS and EU levels.

4.3. Overview on MRSA *spa*-types, sequence types and clonal complexes

In total, 145 MRSA positive pooled dust samples were collected from breeding pig holdings in this survey and 417 MRSA positive pooled dust samples were collected from production holdings with breeding pigs. All MRSA isolates were *spa*-typed, except one originating from the Czech Republic, which had been confirmed as MRSA but did not grow upon sub-cultivation and therefore could not be typed. MRSA isolates were assigned to a ST and a CC, mainly by performing MLST-typing of selected isolates as well as by comparing *spa*-types with databases of fully described strains, deriving from earlier studies. In general, at least one isolate of each *spa*-type was submitted to MLST-typing.

Spa-typing identified 37 *spa*-types among the MRSA isolates gathered from the holdings with breeding pigs involved in the survey. Those *spa*-types belonged to eight STs and eight CCs (Table 2). Fifteen different *spa*-types were isolated from dust samples of breeding pig holdings and 34 different *spa*-types were isolated from dust samples of production holdings with breeding pigs across the EU (Tables 6 and 7).

Most of the MRSA-strains isolated from breeding and production holdings were from *spa*-types associated with ST/CC398 (92.5%). These strains are referred to as MRSA ST398 in this report. A total of 41 isolates in the EU and one from Norway (7.5% of all isolates) were from *spa*-types not related to CC398. These were attributed to 13 different *spa*-types that clustered in seven STs (ST1, ST5, ST8, ST9, ST39, ST97 and ST132). These strains are referred to as MRSA non-ST398 in this report. The non-ST398 isolates were detected in breeding holdings in Italy and in production holdings with breeding pigs in six MSs and Norway. However, the majority (36/42) of non-ST398 isolates originated from Italy and Germany.

Table 2: Translation table from MRSA *spa*-types to sequence types and clonal complexes, MRSA EU baseline survey in breeding pigs, 2008^(a)

MLST-type	Samples with MLST-types	Clonal complex	<i>spa</i> -type	Samples with <i>spa</i> -types	Repeat sequence
ST398	518	CC398	t011	353	08-16-02-25-34-24-25
			t108	60	08-16-02-25-24-25
			t034	42	08-16-02-25-02-25-34-24-25
			t899	20	07-16-23-02-34
			t1197	8	08-16-02-25-46-24-25
			t1451	4	08-16-02-25-34-25
			t567	3	08-02-25-24-25
			t1255	3	08-16-34-24-25
			t1939	3	07-23-02-34
			t2329	3	08-16-159-25-24-25
			t2922	3	07-16-23-34
			t2370	2	08-16-16-02-25-02-25-34-24-25
			t2510	2	08-17-25
			t571	1	08-16-02-25-02-25-34-25
			t1250	1	08-16-02-25-02-25
			t1344	1	08-24-25
			t1456	1	08-16-02-25
			t1457	1	08-16-02-25-34-02-25-34-24-25
			t1793	1	08-16-02-25-02-25-34-24-24-25
			t2330	1	08-16-02-25-34-24-25-25
			t2346	1	08-16-02-25-34-24-24-25
			t3479	1	08-16-02-25-24-24-25
			t4659	1	08-16-02-25-24-24-24-24-25
			t4838	1	283-16-23-02-34
			t4854	1	08-16-02-25-24
			t4872	1	08-16-02-25-34-24-25-34-24-25
ST1	22	CC1	t127	22	07-23-21-16-34-33-13
ST97	14	CC97	t1730	8	26-23-101-21-17-34-34-34-34-33-34
			t426	2	26-23-12-21-17-34-34-34-34-33-34
			t3992	2	26-23-12-21-17-34-34-33-34
			t2112	1	26-23-12-21-17-34-34-34-34-33-34
			t5487	1	26-33-12-12-34-34-33-34
ST39	2	CC30	t007	2	15-12-16-16-16-16-02-25-17
ST5	1	CC5	t002	1	26-23-17-34-17-20-17-12-17-16
ST8	1	CC8	t008	1	11-19-12-21-17-34-24-34-22-25
ST132	1	CC133	t1403	1	03-23-24
ST9	1	CC9	t1430	1	07-16-23-02-12-23-02-34
-	1	-	Non-typeable	1	-

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

4.4. MRSA holding prevalence

4.4.1. MRSA in breeding holdings

The prevalence of MRSA positive breeding holdings in each MS and at EU level as well as in non-MSs are presented in Table 3.

Based on initial single variable regression analysis, no significant effect was detected of the month of sampling and the delay between sampling and testing on the probability of detection of MRSA in a breeding holding. Consequently only non-adjusted prevalence estimates are reported.

4.4.1.1. Prevalence of MRSA positive breeding holdings

MRSA was detected in 12 out of the 24 MSs providing data on breeding holding status (Figure 2). No breeding holdings tested positive in 12 MSs nor in two non-MSs. At MS level, the prevalence was highest in Spain (46.0%) and Germany (43.5%). The EU prevalence was 14.0% (95% CI: 11.7; 16.2). Figure 3 displays the geographic distribution of MRSA prevalence in MSs and other participating countries.

4.4.1.2. Prevalence of MRSA ST398 positive breeding holdings

MRSA ST398 was isolated in all 12 MSs reporting positive results for MRSA in breeding holdings. In 11 of these 12 MSs, all isolates were assigned to this ST. The EU level prevalence was 13.1% (95% CI: 10.9; 15.3). At MS level, the prevalence was highest in Spain (46.0%) and Germany (43.5%) (Table 3 and Figure 4). The geographic distribution of the MRSA ST398 prevalence in MSs and other participating countries is displayed in Figure 5.

4.4.1.3. Prevalence of MRSA non-ST398 positive breeding holdings

The spatial distribution of MRSA non-ST398 differed substantially from the distributions of MRSA (all types) and MRSA ST398. MRSA other than ST398 were only found in breeding holdings from Italy (Table 3), which results in an “EU level” prevalence of 0.8% (95% CI: 0.4; 1.2) (Appendix I).

Table 3: Prevalence of MRSA positive breeding holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	N	MRSA		ST398		Non-ST398	
		%prev.	95%CI ^(b)	%prev.	95%CI ^(b)	%prev.	95%CI ^(b)
Austria	75	5.3	2.5-12.1	5.3	2.5-12.1	0	0.0-4.3
Belgium	15	40.0	21.7-67.1	40.0	21.7-67.1	0	0.0-21.3
Bulgaria	47	0	0.0-4.9	0	0.0-4.9	0	0.0-4.9
Cyprus ^(c)	4	0	0.0-0.0	0	0.0-0.0	0	0.0-0.0
Czech Republic	106	1.9	1.0-5.6	0.9	0.5-4.1	0	0.0-2.6
Denmark	95	0	0.0-3.2	0	0.0-3.2	0	0.0-3.2
Estonia	6	0	0.0-14.3	0	0.0-14.3	0	0.0-14.3
Finland	48	0	0.0-6.1	0	0.0-6.1	0	0.0-6.1
France	157	1.9	0.9-5.0	1.9	0.9-5.0	0	0.0-1.9
Germany	46	43.5	31.6-58.2	43.5	31.6-58.2	0	0.0-7.4
Hungary ^(c)	40	0	0.0-0.0	0	0.0-0.0	0	0.0-0.0
Ireland	40	0	0.0-2.4	0	0.0-2.4	0	0.0-2.4
Italy	43	34.9	24.2-49.5	14.0	7.5-26.3	20.9	12.9-34.4
Latvia	5	0	0.0-28.6	0	0.0-28.6	0	0.0-28.6
Lithuania	10	0	0.0-9.1	0	0.0-9.1	0	0.0-9.1
Luxembourg ^(c)	3	0	0.0-0.0	0	0.0-0.0	0	0.0-0.0
Netherlands	109	12.8	8.4-19.8	12.8	8.4-19.8	0	0.0-3.0
Poland	143	2.1	0.8-6.0	2.1	0.8-6.0	0	0.0-2.5
Portugal	34	14.7	12.8-23.1	14.7	12.8-23.1	0	0.0-5.1
Slovakia	96	1.0	0.7-3.7	1.0	0.7-3.7	0	0.0-2.2
Slovenia	27	11.1	8.6-22.9	11.1	8.6-22.9	0	0.0-8.6
Spain	150	46.0	40.0-52.8	46.0	40.0-52.8	0	0.0-2.2
Sweden	55	0	0.0-5.1	0	0.0-5.1	0	0.0-5.1
United Kingdom	67	0	0.0-3.8	0	0.0-3.8	0	0.0-3.8
European Union ^(d)	1,368 ^(e)	14.0	11.7-16.2	13.1	10.9-15.3	0.8	0.4-1.2
Norway	108	0	0.0-2.2	0	0.0-2.2	0	0.0-2.2
Switzerland	71	0	0.0-3.4	0	0.0-3.4	0	0.0-3.4

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

^(b): 95% CI based on a finite population approach.

^(c): As a census sampling of breeding pig holdings (all existing breeding holdings included in the survey) was performed in Cyprus, Hungary and Luxembourg, the 95% CI based on a finite population approach gave a CI that is equal to the point estimate, although the true CI is likely to be larger.

^(d): EU level prevalence in breeding pig holdings with at least 50 breeding pigs.

^(e): Total number of breeding holdings with at least 50 breeding pigs sampled in the EU.

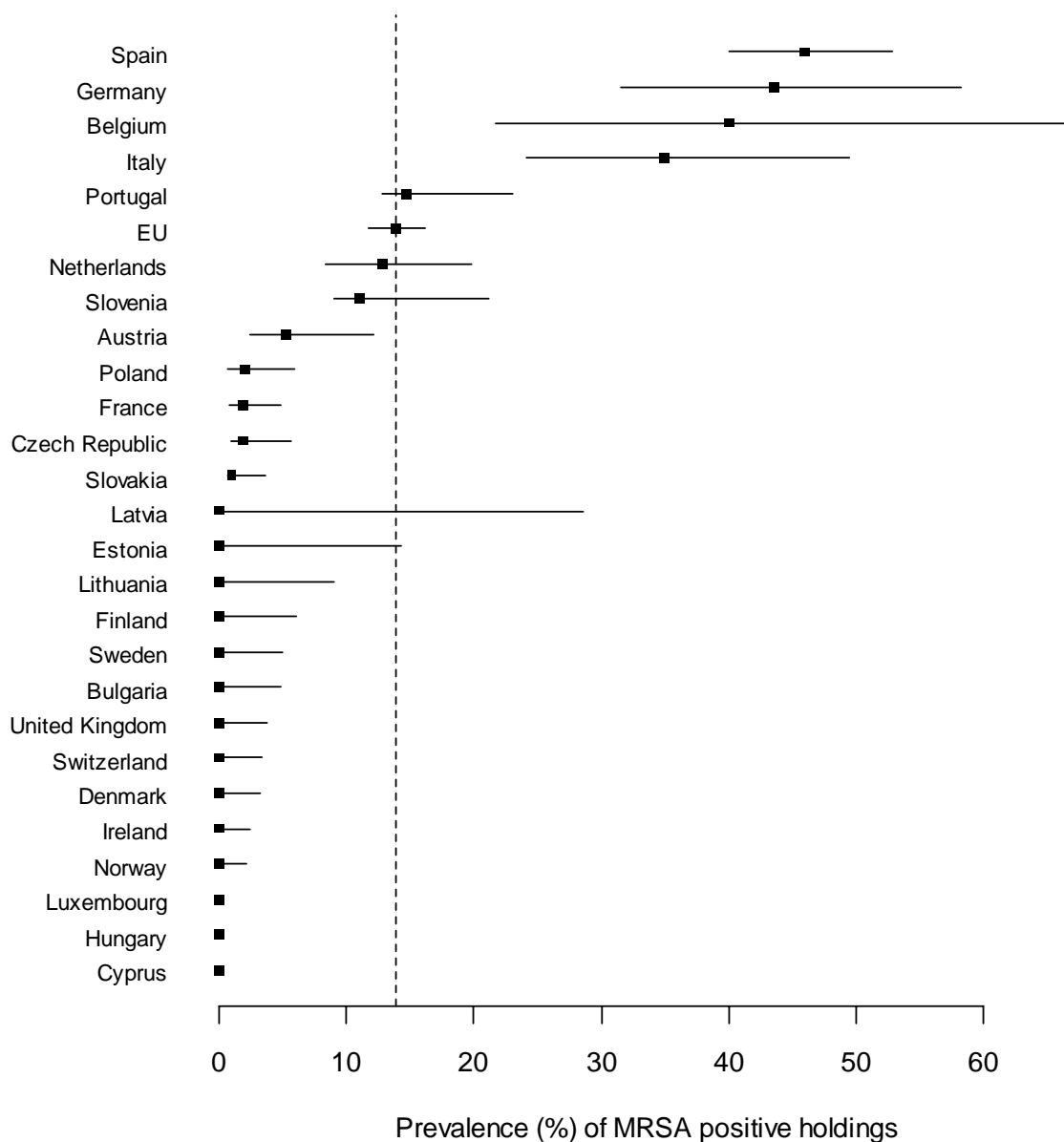


Figure 2: Prevalence^(a) of MRSA positive breeding holdings, with 95% CIs^(b), MRSA EU baseline survey in breeding pigs, 2008^(c)

(a) Horizontal bars represent 95% CIs.

(b) As all existing breeding holdings are included in the survey in Cyprus, Hungary, and Luxembourg (census sampling), a 95% CI based on a finite population approach is equal to the point estimate and therefore no CI is displayed, although the true CI is likely to be larger.

(c) Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

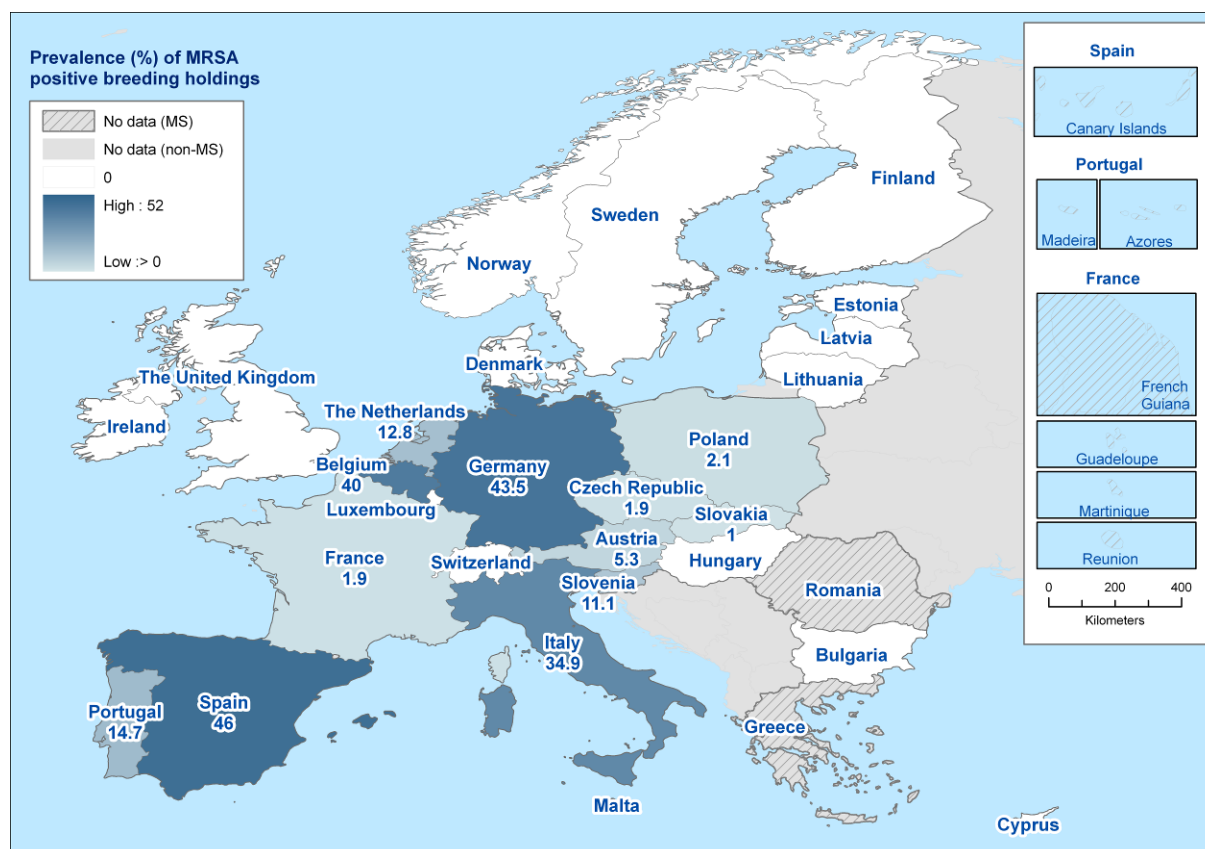


Figure 3: Prevalence of MRSA positive breeding holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

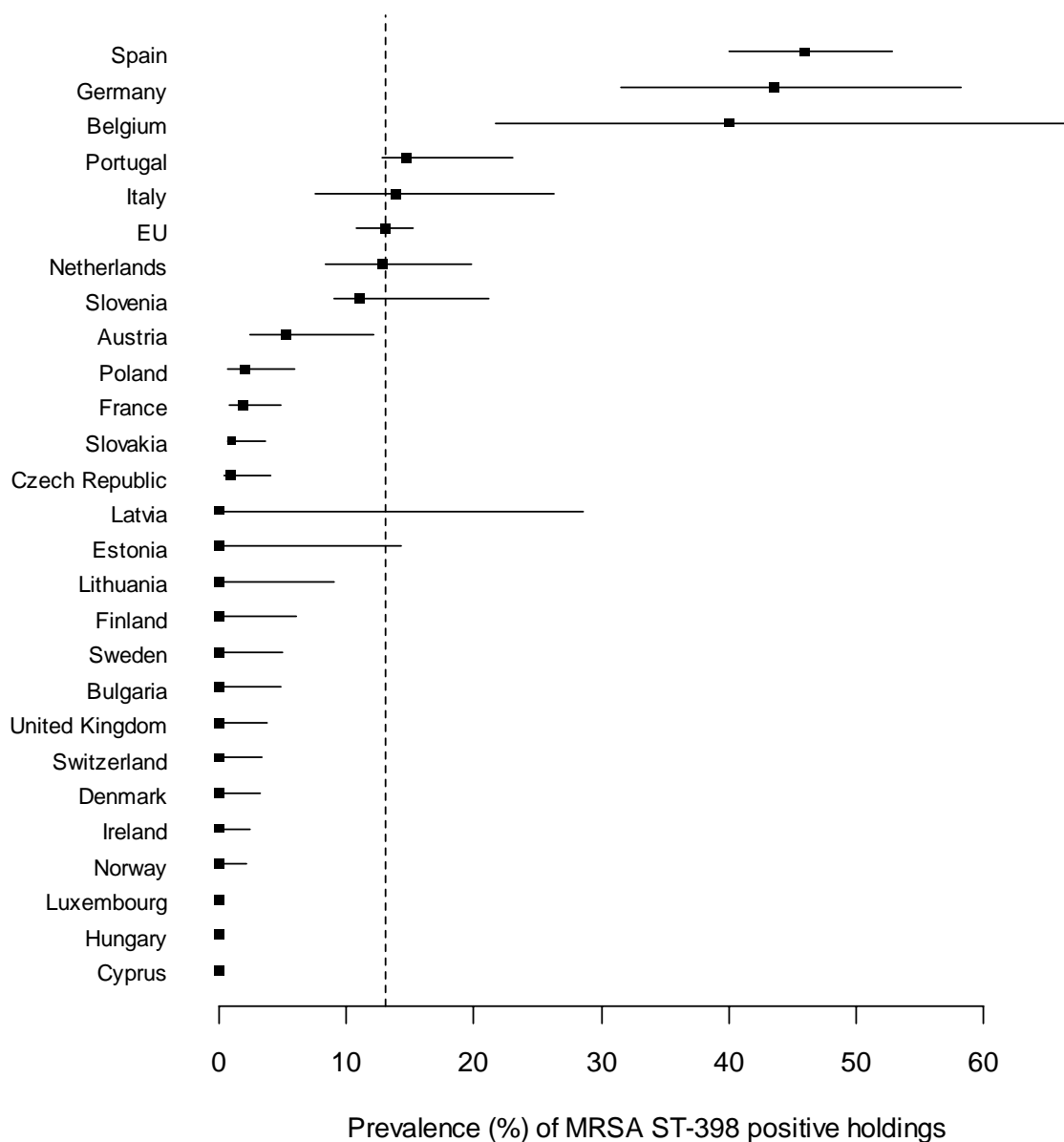


Figure 4: Prevalence^(a) of MRSA ST398 positive breeding holdings, with 95% CIs^(b), MRSA EU baseline survey in breeding pigs, 2008^(c)

(a): Horizontal bars represent 95% CIs.

(b): As all existing breeding holdings were included in the survey in Cyprus, Hungary, and Luxembourg (census sampling), the 95% CI based on a finite population approach is equal to the point estimate and therefore no CI is displayed, although the true CI is likely to be larger.

(c): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

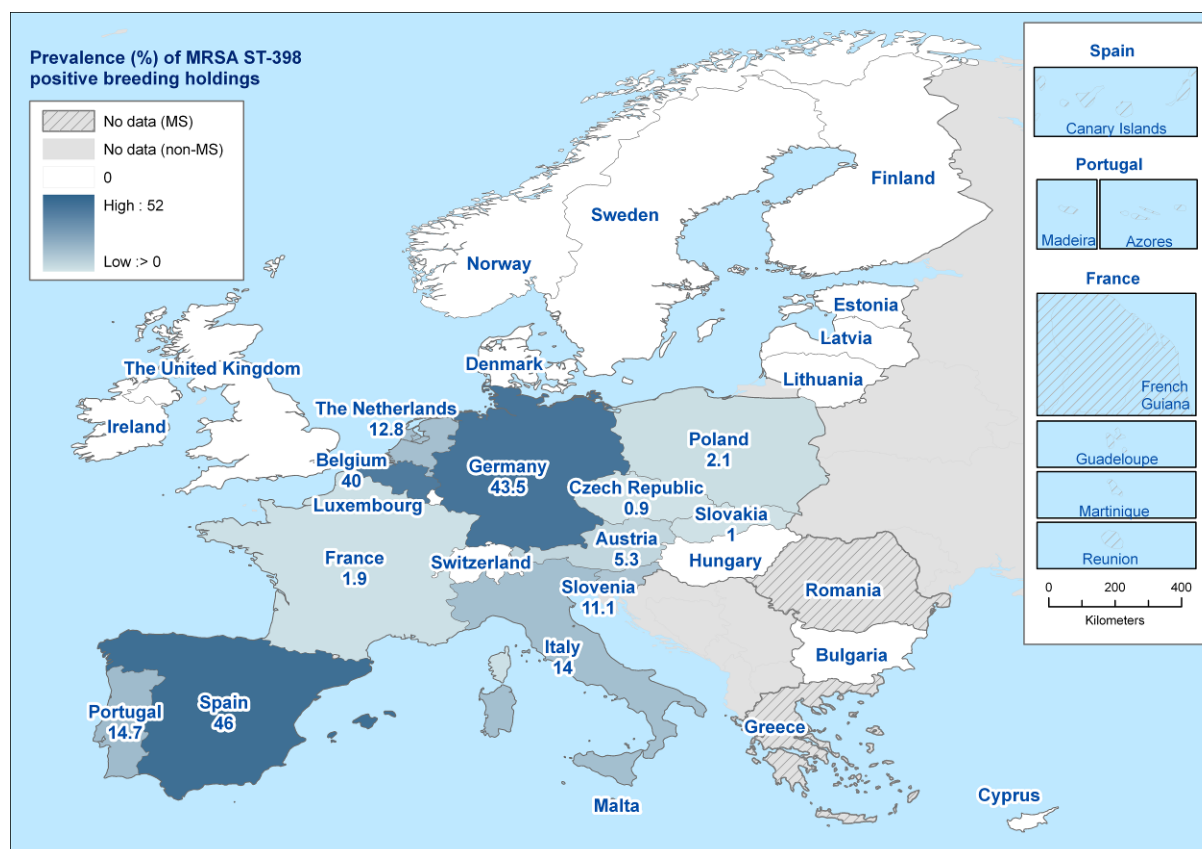


Figure 5: Prevalence of MRSA ST398 positive breeding holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

4.4.2. MRSA in production holdings

The prevalence of MRSA positive production holdings in each of the 24 reporting MSs, and in two non-MSs, as well as at EU level, are presented in Table 4.

As no significant effect of the month of sampling and the delay between sampling and testing was detected on the probability of detection of MRSA positive production holding through initial single variable regression analysis, non-adjusted prevalence estimates were reported at EU and MS level.

4.4.2.1. Prevalence of MRSA positive production holdings

MRSA was detected in 16 out of the 24 MSs providing data on MRSA in production holdings, as well as in Norway (Figure 6). The geographic distribution of prevalence at national level is displayed in Figure 7. No production holdings tested positive in eight MSs and in Switzerland. The EU prevalence was 26.9% (95% CI: 24.4; 29.3). At MS level, prevalence was highest in Spain (51.2%) and Germany (41.3%).

4.4.2.2. Prevalence of MRSA ST398 positive production holdings

MRSA ST398 was isolated in 15 MSs reporting positive results for MRSA in production holdings (Figure 8). No production holdings tested positive in nine MSs, and two non-MSs. The EU level prevalence was 25.5% (95% CI: 23.1; 27.9). At MS level, prevalence was highest in Spain (50.2%) and Germany (37.4%). The geographic distribution of prevalence at national level is displayed in Figure 9.

4.4.2.3. Prevalence of MRSA non-ST398 positive production holdings

MRSA sequence types other than ST398 were found in production holdings from six MSs and Norway. No production holdings tested positive in 18 MSs, and Switzerland. The EU level prevalence was 1.4% (95% CI: 0.6; 2.2). At MS level, prevalence was highest in Italy (12.3%), followed by Germany with 3.9% prevalence; the other countries reported very low or zero prevalence (Figure 10). The geographic distribution of prevalence at national level is displayed in Figure 11.

The EU level prevalence, based on the weighted mean of the MS level prevalences, was notably greater than the proportion of positive production holdings among the sampled holdings. This is due to the fact that several countries with a high prevalence of MRSA positive holdings also had a substantial production holding population compared to other MSs. Indeed, the large number of production holdings in those countries yield limited sampling fractions in those countries and therefore important weight is allocated to the tested holdings in those countries.

An overview of the detection of MRSA, MRSA ST398 and MRSA non-ST398 among the breeding and production holdings in the EU and two non-MSs is displayed in Table 5. Eleven out of 24 MSs detected MRSA in both breeding and production holdings. Only one MS detected MRSA in breeding holdings but not in production holdings, while five MSs and one non-MS only reported MRSA in production holdings. The number of MSs having detected MRSA ST398 in breeding and production holdings was the same as that of MSs having reported MRSA. Conversely, the picture of MRSA non-ST398 detection in the EU was different. Indeed, only one MS detected MRSA non-ST398 in breeding holdings, while six MSs and one non-MS detected MRSA non-ST398 in production holdings.

Table 4: Prevalence of MRSA positive production holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	N	MRSA		ST398		non-ST398	
		%prev.	95%CI ^(b)	%prev.	95%CI ^(b)	%prev.	95%CI ^(b)
Austria	167	12.6	8.5-18.5	12.6	8.5-18.5	0	0.0-2.2
Belgium	184	35.9	29.6-43.1	35.9	29.6-43.1	0	0.0-2.0
Bulgaria	25	0	0.0-13.5	0	0.0-13.5	0	0.0-13.5
Cyprus	65	1.5	1.1-5.7	0	0.0-3.4	1.5	1.1-5.7
Czech Republic	161	1.2	0.4-4.4	1.2	0.4-4.4	0	0.0-2.2
Denmark	198	3.5	1.8-7.1	3.5	1.8-7.1	0	0.0-1.8
Estonia ^(c)	28	0	0.0-0.0	0	0.0-0.0	0	0.0-0.0
Finland	150	0.7	0.1-3.4	0.7	0.1-3.4	0	0.0-2.3
France	185	2.7	1.2-6.2	2.2	0.9-5.4	0.5	0.1-3.0
Germany	155	41.3	34.1-49.4	37.4	30.4-45.5	3.9	1.8-8.2
Hungary	141	2.1	0.8-5.6	2.1	0.8-5.6	0	0.0-2.3
Ireland	149	0	0.0-2.0	0	0.0-2.0	0	0.0-2.0
Italy	171	33.9	27.5-41.4	21.6	16.3-28.5	12.3	8.3-18.1
Latvia	28	0	0.0-7.7	0	0.0-7.7	0	0.0-7.7
Lithuania	72	0	0.0-2.4	0	0.0-2.4	0	0.0-2.4
Luxembourg ^(c)	41	36.6	36.6-36.6	36.6	36.6-36.6	0	0.0-0.0
Netherlands	212	18.4	14.0-24.1	17.9	13.6-23.6	0.5	0.1-2.5
Poland	178	3.4	1.6-7.2	3.4	1.6-7.2	0	0.0-2.0
Portugal	136	11.8	7.5-18.3	11.8	7.5-18.3	0	0.0-2.6
Slovakia	96	0	0.0-3.7	0	0.0-3.7	0	0.0-3.7
Slovenia	87	5.7	2.6-12.9	5.7	2.6-12.9	0	0.0-4.1
Spain	209	51.2	44.7-58.1	50.2	43.8-57.2	1.0	0.3-3.4
Sweden	147	0	0.0-2.4	0	0.0-2.4	0	0.0-2.4
United Kingdom	191	0	0.0-1.8	0	0.0-1.8	0	0.0-1.8
European Union ^(d)	3,012 ^(e)	26.9	24.4-29.3	25.5	23.1-27.9	1.4	0.6-2.2
Norway	143	0.7	0.2-3.7	0	0.0-2.5	0.7	0.2-3.7
Switzerland	154	0	0.0-2.2	0	0.0-2.2	0	0.0-2.2

(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

(b): 95% CI based on a finite population approach.

(c): As a census sampling of production holdings with breeding pigs (all existing holdings of this type included in the survey) was performed in Estonia and Luxembourg, the 95% CI based on a finite population approach gave a CI that is equal to the point estimate, although the true CI is likely to be larger.

(d): EU level prevalence in production holdings with at least 50 breeding pigs.

(e): Total number of production holdings with at least 50 breeding pigs sampled in the EU.

Table 5: Overview of the detection of MRSA in breeding and production holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	MRSA		ST398		non-ST398	
	Breeding holdings	Production holdings	Breeding holdings	Production holdings	Breeding holdings	Production holdings
Austria	+	+	+	+	-	-
Belgium	+	+	+	+	-	-
Bulgaria	-	-	-	-	-	-
Cyprus	-	+	-	-	-	+
Czech Republic	+	+	+	+	-	-
Denmark	-	+	-	+	-	-
Estonia	-	-	-	-	-	-
Finland	-	+	-	+	-	-
France	+	+	+	+	-	+
Germany	+	+	+	+	-	+
Hungary	-	+	-	+	-	-
Ireland	-	-	-	-	-	-
Italy	+	+	+	+	+	+
Latvia	-	-	-	-	-	-
Lithuania	-	-	-	-	-	-
Luxembourg	-	+	-	+	-	-
Netherlands	+	+	+	+	-	+
Poland	+	+	+	+	-	-
Portugal	+	+	+	+	-	-
Slovakia	+	-	+	-	-	-
Slovenia	+	+	+	+	-	-
Spain	+	+	+	+	-	+
Sweden	-	-	-	-	-	-
United Kingdom	-	-	-	-	-	-
Norway	-	+	-	-	-	+
Switzerland	-	-	-	-	-	-

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

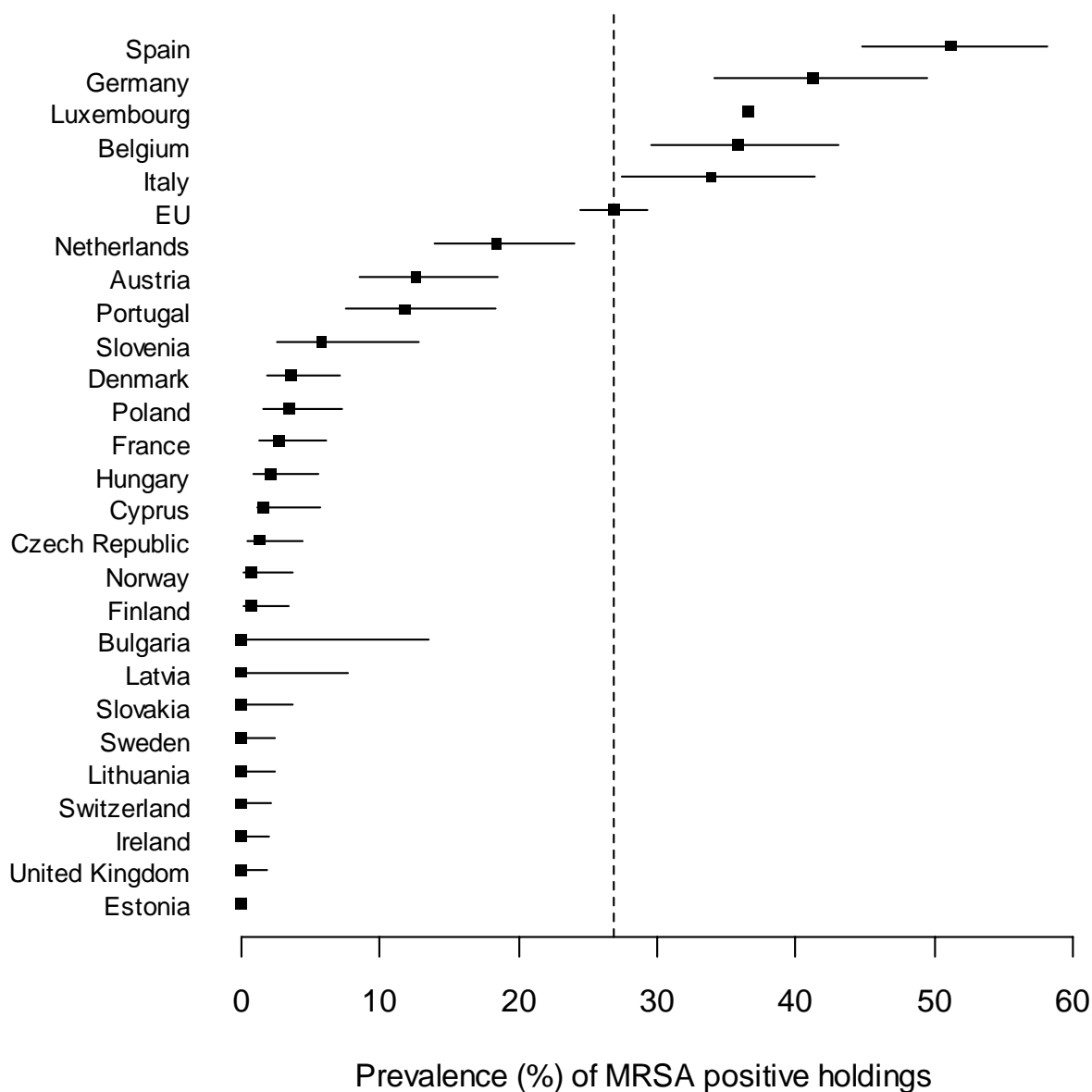


Figure 6: Prevalence^(b) of MRSA positive production holdings, with 95% CIs^(c), MRSA EU baseline survey in breeding pigs, 2008^(a)

(a): Horizontal bars represent 95% CIs.

(b): As all existing breeding holdings were included in the survey in Estonia and Luxembourg (census sampling), the 95% CI based on a finite population approach is equal to the point estimate and therefore no CI is displayed, although the true CI is likely to be larger.

(c): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.



Figure 7: Prevalence of MRSA positive production holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

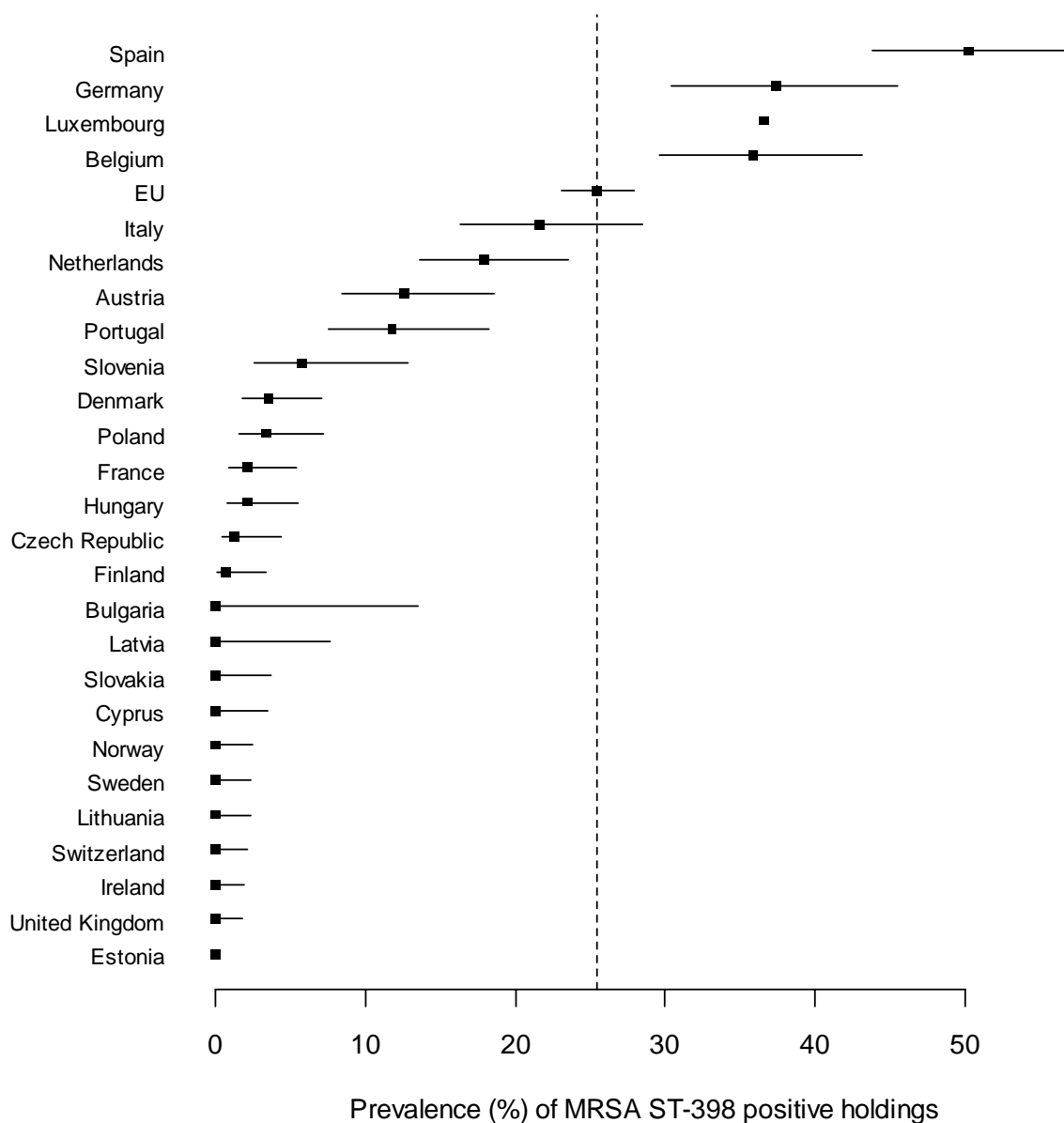


Figure 8: Prevalences^(a) of MRSA ST398 positive production holdings, with 95% CIs^(b), MRSA EU baseline survey in breeding pigs, 2008^(c)

(a): Horizontal bars represent 95% CIs.

(b): As all existing breeding holdings were included in the survey in Estonia and Luxembourg (census sampling), the 95% CI based on a finite population approach is equal to the point estimate and therefore no CI is displayed, although the true CI is likely to be larger.

(c): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

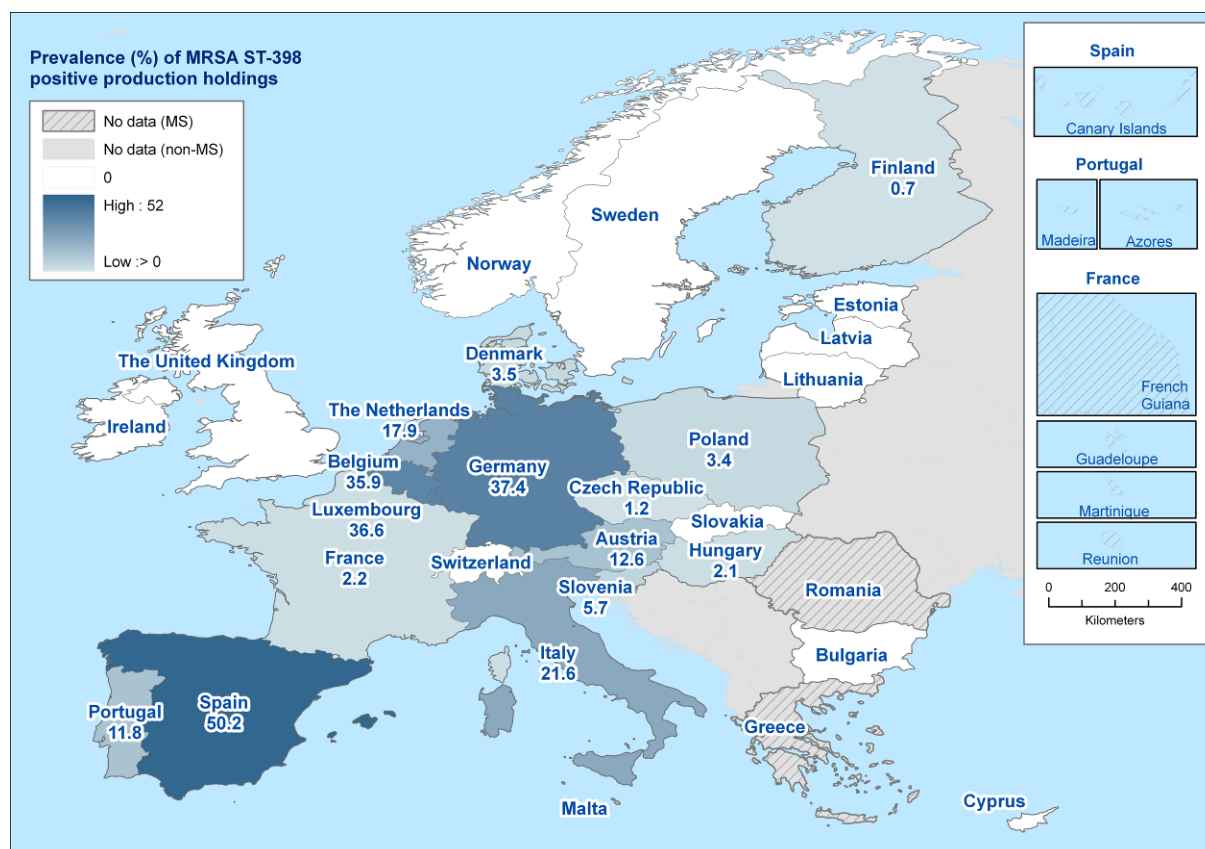


Figure 9: Prevalence of MRSA ST398 positive production holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

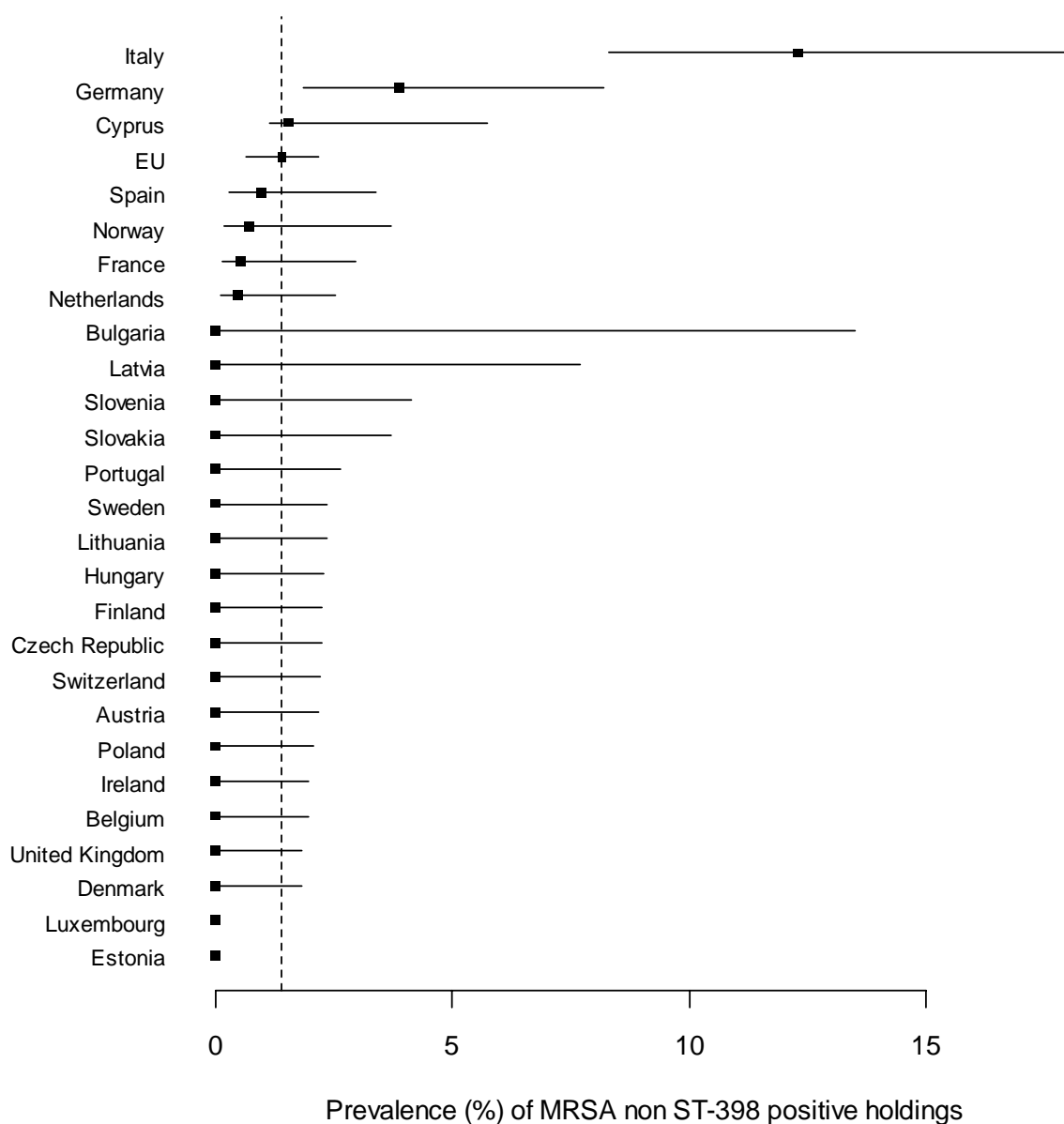


Figure 10: Prevalences^(a) of MRSA non-ST398 positive production holdings, with 95% CIs^(b), MRSA EU baseline survey in breeding pigs, 2008^(c)

(a): Horizontal bars represent 95% CIs.

(b): As all existing breeding holdings were included in the survey in Estonia and Luxembourg (census sampling), the 95% CI based on a finite population approach cannot be calculated and therefore no CI is displayed, although the true CI is likely to be larger.

(c): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

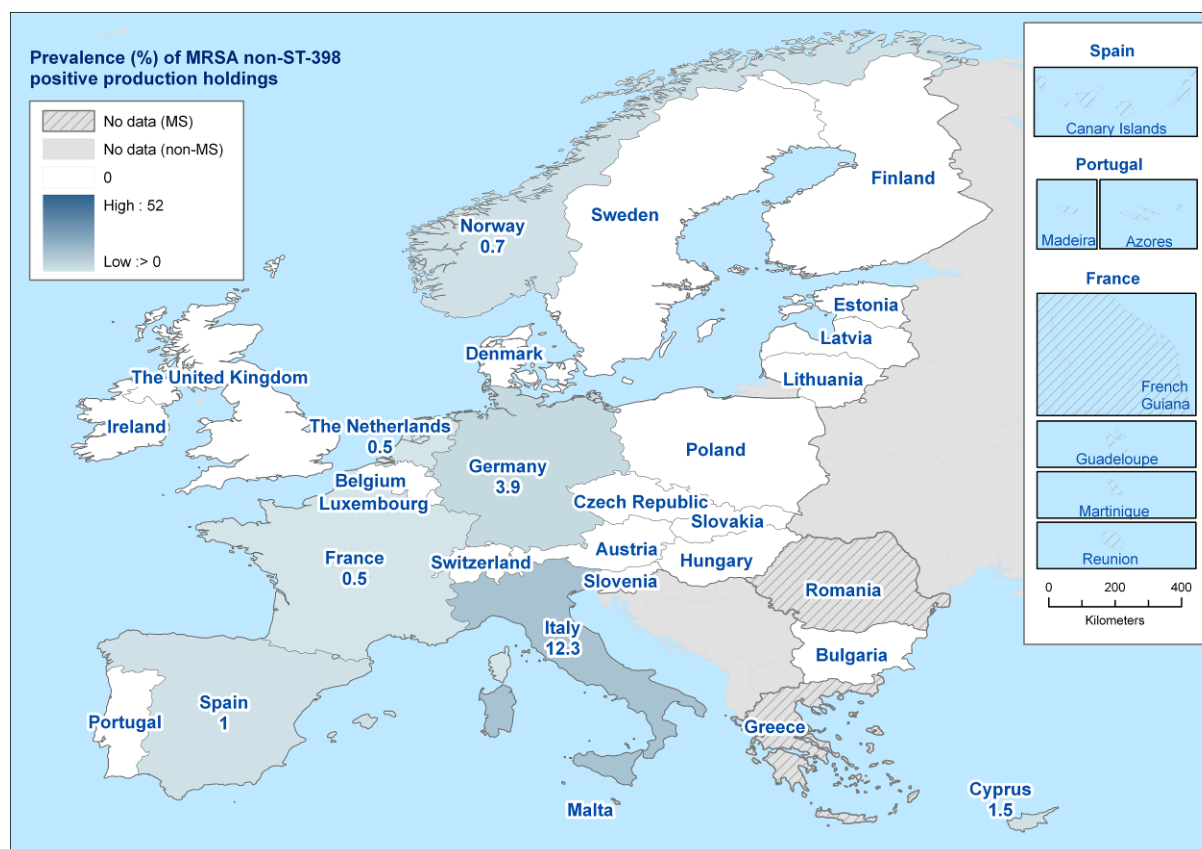


Figure 11: Prevalence of MRSA non-ST398 positive production holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

4.4.3. MRSA in holdings with breeding pigs

In this survey, the EU prevalence of MRSA positive holdings with breeding pigs (all holdings, including both breeding and production holdings) with at least 50 breeding pigs was 22.8% (95% CI: 21.0; 24.7). This means that about one in four holdings with breeding pigs was positive for MRSA. However, prevalence varied among MSs, from 0% to 49%. The EU prevalence of MRSA ST398 and of MRSA non-ST398 in the holdings were respectively, 21.6% (95% CI: 19.8; 23.4) (MS prevalence range from 0% to 48.5%) and 1.2% (95% CI: 0.7; 1.8) (MS prevalence range from 0% to 14%). However, while 17 MSs detected MRSA in their holdings with breeding pigs, seven MSs did not find MRSA in their holdings within the survey.

5. Frequency distribution of MRSA *spa*-types

The frequency distributions of isolated MRSA *spa*-types in breeding holdings and production holdings from the EU, and the two non-MSs are listed in Table 6 and Table 7, respectively. MS-specific overviews of the frequency distribution of *spa*-types are shown in Appendix G (Tables 16 and 17). The Table 2 provides for the translation from MRSA *spa*-types to sequence types (ST) and clonal complexes (CC).

The t011 *spa*-type was by far the most dominant type found among the isolates in the baseline survey. It was found among 60.7% of the MRSA isolates from breeding pig holdings and 63.5% of the MRSA isolated from production holdings with breeding pigs. Also, *spa*-types t108 and t034 were found in relatively high numbers in both holding populations (Tables 6 and 7). All three *spa*-types are associated with MRSA ST398/CC398 (Table 2). The *spa*-types t011, t034 and t108 were clearly the most dominant, accounting for 81.0% of all strains isolated and the most frequent *spa*-types in nearly all MSs that isolated MRSA from holdings. Other *spa*-types of the same CC398 were less frequent in the survey. However, together they accounted for 11.4 % of all MRSA isolates. In breeding holdings their proportion was 13.1%, in production holdings, with breeding pigs it was 11.0%.

Most of the remaining MRSA isolates were *spa*-type t127 which belongs to ST1/CC1, or *spa*-type t1730 belonging to ST97/CC97. Of the isolates with *spa*-type t127, 85% (n=19) originated from Italy, 10% (n=2) from Spain, and 5% from Cyprus (n=1), while 85% (n=11) of the isolates with *spa*-types related to ST97/CC97 originated from Italy and 15% (n=3) from Germany.

The *spa*-type t899 that was assigned to ST/CC398 by MLST-typing was only present in Italy and France. It had a *spa* repeat distribution that would predict the isolates belonging to CC9 rather than to CC398. However, MLST typing of a sub-set of the t899 isolates revealed that they in fact belonged to ST398/CC398. As the t899 isolates were typed to ST398/CC398, the overall result of the clonal investigation was that 69% of the *spa*-types detected, containing 92.5% of the isolates, belonged to ST398/CC398.

Interestingly, one MRSA isolate from Germany had *spa*-type t1430. This *spa*-type is related to t899 but was MLST typed to ST9/CC9 in this survey.

Table 6: Frequency distribution of isolated *spa*-types of MRSA in breeding holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

<i>spa</i> -type	Samples with <i>spa</i> -types		Countries with <i>spa</i> -types
	N	%	N
t011	88	60.7	9
t108	21	14.5	4
t034	7	4.8	5
t127	5	3.4	1
t899	5	3.4	2
t1197	4	2.8	1
t1730	4	2.8	1
t1255	2	1.4	1
t2329	2	1.4	1
t1344	1	0.7	1
t1456	1	0.7	1
t2370	1	0.7	1
t2510	1	0.7	1
t2922	1	0.7	1
t4659	1	0.7	1
Non-typeable	1	0.7	1
	145	100.0	-

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

Table 7: Frequency distribution of isolated *spa*-types of MRSA on production holdings with breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(a)

<i>spa</i> -type	Samples with <i>spa</i> -types		Countries with <i>spa</i> -types
	N	%	N
t011	265	63.5	12
t108	39	9.4	6
t034	35	8.4	10
t127	17	4.1	3
t899	15	3.6	2
t1197	4	1.0	1
t1451	4	1.0	3
t1730	4	1.0	1
t567	3	0.7	2
t1939	3	0.7	1
t007	2	0.5	1
t426	2	0.5	1
t2922	2	0.5	1
t3992	2	0.5	1
t002	1	0.2	1
t008	1	0.2	1
t571	1	0.2	1
t1250	1	0.2	1
t1255	1	0.2	1
t1403	1	0.2	1
t1430	1	0.2	1
t1457	1	0.2	1
t1793	1	0.2	1
t2112	1	0.2	1
t2329	1	0.2	1
t2330	1	0.2	1
t2346	1	0.2	1
t2370	1	0.2	1
t2510	1	0.2	1
t3479	1	0.2	1
t4838	1	0.2	1
t4854	1	0.2	1
t4872	1	0.2	1
t5487	1	0.2	1
Total isolates	417	100.0	-

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

5.1. Phylogenetic analysis of the relationship between the *spa*-types isolated

The phylogenetic analysis was performed on all *spa*-typed MRSA isolates available in the survey. *Spa*-typing data were analysed for all holdings with breeding pigs combined (Figure 12), as well as for breeding pig holdings and production units with breeding pigs (Figures 25 and 26 in Appendix H), separately. The minimal Spanning tree algorithm was used to calculate population modelling networks in cluster analysis and therefore enabled the organisation of closely related *spa*-types into larger *spa*-type groups. Output figures display MRSA isolates, grouped first by *spa*-types represented by circles whose sizes are proportionate to the number of isolates identified for each specific *spa*-type. By the phylogenetic analysis, isolated strains have also been organised into CCs of closely related strains that share alleles in common. Distance coding between the different *spa*-types is also displayed on the output figures. Two *spa*-types connected to each other by a thick line are more closely related to each other than *spa*-types connected with a thin line. *Spa*-types, which are not connected by any lines, are less related and will most often belong to different CCs. Finally, *spa*-type groups have been categorised according to their belonging to either the ST398 or the non-ST398 group, based on the result of subsequent MLST typing of representative isolates from each *spa*-type. The Table 2 provides for the translation from MRSA *spa*-types to sequence types (ST) and clonal complexes (CC).

In general, most of the *spa*-types identified in breeding pig holdings were also identified in production holdings with breeding pigs, as only three *spa*-types (t1344, t1456, t4659) were unique for breeding pig holdings representing only 2.1% of the isolates, and all three *spa*-types belonged to ST398. By comparing the *spa*-typing data obtained from breeding pig holdings with those obtained from production holdings (Appendix H), it was evident that there was a higher diversity of *spa*-types in production holdings than in breeding holdings, but this may be explained by the fact that more samples were collected in production holdings.

Caption of Figure 12

Colour code

- Red: >20 isolates with the same *spa*-type
- Dark red: between 10 and 20 isolates with the same *spa*-type
- Dark blue: between 6 and 10 isolates with the same *spa*-type
- Blue: between 3 and 5 isolates with the same *spa*-type
- Light blue: 1 or 2 isolates with the same *spa*-type

Distance coding

- Thick black line: 1-2 changes* between *spa*-types
- Thin black line: 3-4 changes* between *spa*-types

* Changes are equal to changes in repeats. This can be a change in one *spa* repeat (substitution of point mutation or a deletion/addition of a *spa* repeat).

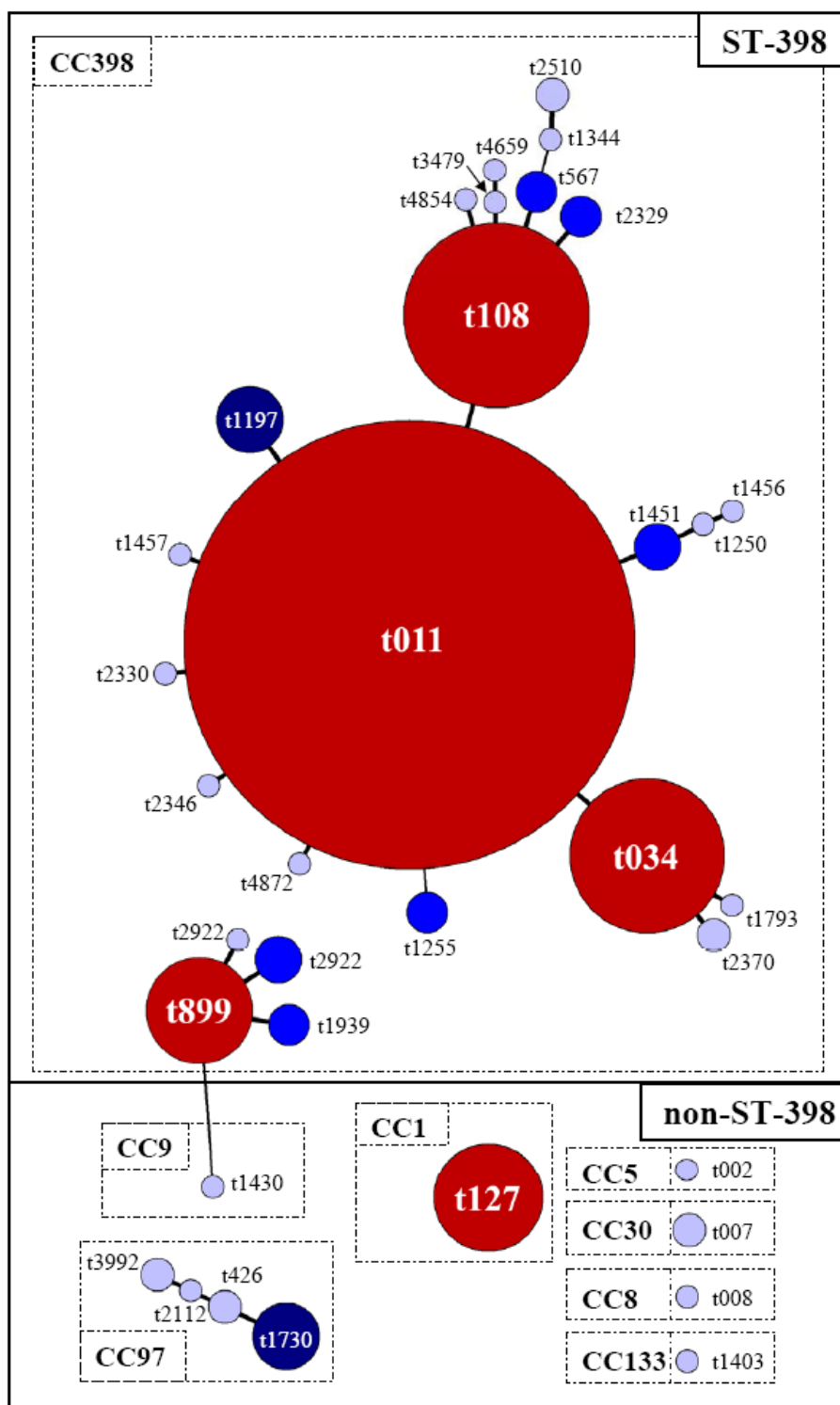


Figure 12: Phylogenetic analysis^(a) of the relationship between the *spa*-types isolated in breeding and in production holdings, MRSA EU baseline survey in breeding pigs, 2008^(b)

- (a): For analysis, the minimal Spanning tree algorithm was used with the settings: gap creation cost: 50%; gap extension cost: 50%; duplication creation cost: 25%; duplication extension: 25%; maximum duplication length: 3 re; bin group distance: 50%.
- (b): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

5.2. Overview of the quality of the MRSA testing

The CRL-AR was responsible for the quality assurance (QA) of the identification of isolates obtained by MSs in the context of the MRSA baseline survey in holdings with breeding pigs. QA testing was performed on up to 16 MRSA isolates from each MS with positive herds as described in Annex I, part C (2.3.2) of Commission Decision 2008/55/EC. MSs were also encouraged to submit presumptive *S. aureus* isolates which were not identified as MRSA (non-MRSA). In total, 136 MRSA and only 36 presumptive MRSA not identified as MRSA (non-MRSA) were submitted by MSs. However, as the culture procedure utilised selective stages to filter out non-MRSA, it is unsurprising that some MSs recovered no *S. aureus* isolates which were not MRSA. Species identification and presence of the *mecA* gene were re-tested on all isolates at the CRL-AR using a multiplex PCR with simultaneous identification of the *mecA*-gene, the *nuc* gene, and the 16S *rrn* ribosomal sub-unit. Re-testing confirmed that all MSs that had submitted strains for QA had correctly identified MRSA isolates as MRSA (no false positive results) and none of the 36 non-MRSA was identified as MRSA upon re-testing (no false negative results). However, four MSs, who had reported positive isolates for MRSA, did not submit isolates for re-testing to CRL-AR.

6. Discussion

6.1. Context and strength of the MRSA baseline survey

Information related to the prevalence of MRSA in pigs at EU and MS levels was previously identified in 2007 in EFSA's report on a proposal for technical specifications of the MRSA baseline survey (EFSA, 2007). This was identified as one of the preliminary key pieces of information required in pigs in Europe to investigate the existence of a clonal spread of MRSA ST398, the strain diversity of MRSA in that species, as well as the significance of the occurrence of MRSA in pigs in relation to the overall occurrence of MRSA strains in humans. In order to gather preliminary data on the prevalence of MRSA in pig holdings in MSs at the earliest opportunity, the preliminary survey on the prevalence of MRSA in breeding pigs was linked to a baseline survey on the prevalence of *Salmonella* in breeding pigs, which was at an advanced planning stage. This had several advantages of using a representative sample of pig holdings, which had already been identified in the baseline *Salmonella* survey, and also of minimising the additional burden on MSs as MRSA sampling could be completed during the visit to perform the *Salmonella* investigation.

The preliminary survey on MRSA in breeding pigs was set up as a Community baseline survey and has provided the first indication of comparable holding level prevalence of MRSA in breeding pigs at MS and at EU level. The epidemiological units of the survey were holdings with breeding pigs, mainly those with at least 50 breeding pigs, regardless of the holding size, i.e. the number of sows or the number of pigs produced or the number of pig workers employed. The survey protocol for farm investigation, microbiological culture techniques and molecular techniques used for definitive identification and typing of MRSA were harmonised and used by all MSs. The use of a standardised sampling and testing protocol should therefore ensure that results are, as far as possible, comparable between MSs.

Five environmental dust samples from each pig holding with breeding pigs were collected and examined pooled during the survey. Other sampling methods might have been used to gather relevant samples to investigate the MRSA status of a pig herd (e.g. 60 nasal swabs per holding). However, at the time when the survey was planned, it was acknowledged (EFSA, 2007) that taking five dust samples on large swabs was only marginally less sensitive than collecting 60 nasal swabs per holding and provided a considerable advantage in terms of ease of sampling, time constraints, and resource requirements. However, it should be noted that the survey objective was to estimate the prevalence of colonised holdings while acknowledging that the cut-off for declaring a holding infected, based on the used survey test, remains to be investigated. For outdoor holdings, it was suggested that dust should be

collected from indoor facilities in those holdings, where those were present. Dust samples were collected in accordance with a standardised protocol, from specified locations, to try to maximise the comparability of the results.

The isolation procedure involved an enrichment Mueller-Hinton broth stage with 6.5% NaCl, then a selective TSB stage containing cefoxitin and aztreonam, followed by plating onto a chromogenic MRSA-selective medium. This procedure, involving both enrichment and selective broth stages, would be expected to detect low levels of MRSA, although the limit of detection of MRSA in dust is unknown. A standardised procedure was adopted for examining suspect MRSA colonies from culture plates, involving up to five colonies (EFSA, 2007).

The survey stipulated that isolates presumptively identified as *S. aureus* or MRSA should be subject to molecular confirmation. This molecular confirmation included both confirmation that bacterial identification was correct (i.e. that the isolate was *S. aureus* and not another *Staphylococcus*) and that the *mecA* gene habitually carried by MRSA was present. This molecular confirmation of the identification of *S. aureus* and of the *mecA* gene provided an extremely high degree of certainty in the positive identification of MRSA from samples. The isolation and identification protocol is therefore considered as highly successful in terms of specificity.

The pig holdings sampled by MSs comprised a representative sample of the breeding pig holdings and of the production holdings and it is therefore valid to extrapolate prevalence results to the respective holding populations from which the representative samples were derived. However, result data on sampled production holdings, in which only the herd of breeding pig was tested for MRSA, cannot be extrapolated to fattening pig holdings in general (i.e. weaner-to-finish holdings and finisher holdings). Future investigation and monitoring of the occurrence and diversity of MRSA in fattening pigs might therefore be of interest, as it may well be in other animal species, since MRSA has also been detected in poultry, cattle, and horses (EFSA, 2009).

6.2. MRSA prevalence in holdings with breeding pigs

The EU MRSA prevalence was estimated on the basis of the results from the 24 participating MSs. MRSA was commonly detected in holdings with breeding pigs in the survey. The EU prevalence of 22.8% was high, even though one-third of the MSs did not find any holdings positive to MRSA. Indeed the disparity of the MRSA prevalence among the MSs was large. In six MSs the MRSA prevalence in the holdings was high, and the two MSs reporting the highest MRSA prevalence are the ones having the biggest pig population in the EU, which emphasises the importance of the findings.

The MS level prevalence estimate is chiefly representative of holdings with at least 50 breeding sows. Even though holdings with a greater number of breeding pigs are likely to pose a higher risk of infection for fattening holdings with MRSA, the prevalence estimate for the individual countries could not take holding size into account, because neither the exact number of breeding pigs nor the within-holding prevalence of MRSA were known. Therefore, the weighted EU prevalence gives a greater weight to countries with a larger number of holdings, but not to countries with a larger number of sows within holdings. Thus, the prevalence is not likely to be representative of the proportion of MRSA colonised breeding pigs in a country or in the EU. Similarly, prevalence estimates do not necessarily reflect the pig worker exposure, as the number of pig handlers per holding may presumably be proportionate to the holding size (i.e. number of breeding pigs).

It is important to note that the absence of any MRSA from the tested samples in this survey does not imply that a MS is MRSA-free in the holding types investigated, as the detection method has an imperfect sensitivity, so false negative results may have presumably occurred. Indeed, testing herds with the objective of certifying herds or zones as colonisation-free would have required a specific screening approach.

MRSA ST398 is by far the most prevalent clone of MRSA in holdings with breeding pigs in the EU, and the MRSA ST398 prevalence pattern parallels that for MRSA. Overall, the findings of the present survey demonstrate the heterogeneity of the situation between EU MSs. Explanatory factors of such a heterogeneous situation should be investigated further.

6.2.1. In breeding holdings

The variation among MRSA prevalence estimates in breeding holdings in MSs was considerable, between 0% and 46%. MRSA was not detected in 12 of the 24 MSs providing data and neither of the two non-MSs. MRSA prevalence was less than 5% in four out of 12 MSs where MRSA was detected. At EU level, MRSA prevalence in breeding holdings was assessed through a weighted mean at 14%, with a 95% CI 11.7% - 16.2%. Except for one MS, the MRSA strains isolated from breeding holdings only belonged to sequence type ST398. In this particular MS, MRSA non-ST398 strains were more prevalent than MRSA ST398 isolates in their breeding holdings. Further investigations should be performed to find the source(s) from which this MRSA non-ST398 originates, as non-ST398 seems uncommon in breeding pigs in the framework of this survey.

The relevance of MRSA infection in breeding holdings is mainly related to the potential for vertical transmission to production holdings with breeding pigs. Breeding pig holdings are at the top of the production pyramid and the presence of MRSA in breeding herds may contribute to a significant level of infection of production holdings with breeding herds. The correlation between prevalence in breeding holdings and production holdings will be investigated at country level in report part B.

6.2.2. In production holdings with breeding pigs

In this survey, MRSA prevalence was higher within production holdings than in breeding holdings. The EU prevalence of MRSA in production holdings was assessed at 26.9%, with a 95% CI 24.4% – 29.3%. Disparity exists between MSs, with eight MSs and one non-MS reporting no detected MRSA in those production holdings. In the 16 MSs, and one non-MS that reported MRSA in production holdings, prevalence ranged from 0.7% up to 51.2%. It was mainly the same MSs that reported the highest MRSA prevalence in breeding and production holdings.

In analogy with breeding holdings, the contribution of MRSA ST398 to EU prevalence was highly predominant compared to that of non-ST398, 25.5% and 1.4%, respectively. Of the six MSs and one non-MS, reporting non-ST398, only four MSs reported a prevalence higher than or equal to 1%, one of which being as high as 12.3%.

The relevance of MRSA infection in production holdings, apart from transferring the infection to pig workers, is related to the potential for vertical transmission to fattening pigs and holdings with growing pigs only (weaner-to-finish and finisher holdings). Holdings with fattening pigs only are numerous in MSs and therefore the risk for transfer to humans could be augmented.

6.3. Impact of test misclassification on prevalences

For the isolation of MRSA from dust, one preliminary study (EFSA, 2007) was available at the start of this survey. This study carried out in the Netherlands indicated that taking five dust samples was only marginally less sensitive than the examination of 60 nasal swabs. However, no gold standard exists for the detection of MRSA infection in pigs. It must therefore be assumed that true test sensitivity is below 100%. The lack of dust to collect in certain types of breeding pig facilities, and the pooling of swabs, instead of analysing them separately, may presumably have contributed to a lower sensitivity of the test. However, the specificity of the method was assumed to be 100%, because a series of confirmatory tests were performed on each presumable MRSA isolate. Therefore, due to the nature of

the test (highly specific but most probably missing some sensitivity), the true prevalence of MRSA positive holdings are likely to be greater than the prevalence observed in this survey.

The survey provides data on the minimum prevalence of MRSA in dust from breeding pig holdings in MSs and at Community level. The work from the Netherlands (EFSA, 2007) suggests that there is a strong correlation between the presence of MRSA ST398, the most prevalent ST in this survey, in dust and the presence of MRSA ST398 in pigs, on a holding. The survey has also detected other STs of MRSA in dust and it is assumed that this relation also holds true for these other STs. Thus, detection of MRSA of a specific type in dust on a holding is assumed to correlate with the presence of that type of MRSA in pigs; however that assumption has only been investigated for MRSA ST398.

The possibility that herds positive for one MRSA lineage might contain other MRSA lineages (possibly at a lower prevalence) was not addressed by the survey protocol, as only one MRSA isolate detected was further characterised. However, reference to the published European literature concerning farm-based studies suggests that this scenario is likely to be uncommon (van Duinkerken et al. 2008, Köck et al. 2009).

6.4. Diversity of MRSA in holdings with breeding pigs

All MRSA strains isolated in the framework of the survey were *spa*-typed and related STs were subsequently assigned to each MRSA isolate, either through performing MLST-typing for new strains or checking relevant databases for strains already described. Molecular typing of MRSA isolates has been completed by the NRLs and the CRL-AR and the typing methods used (*spa*-typing and MLST) have been harmonised and standardised, again facilitating direct comparison of the strains of MRSA detected by MSs.

Spa-typing is a quick and useful tool for characterising MRSA strains, as the method is objective and therefore suitable for the comparison of strains between regions or countries. It has been described that isolates with one given *spa*-type are linked to closely related STs (Harmsen, et al., 2003). At the same time, several *spa*-types are commonly found associated to one ST (or CC) (Deurenberg, et al., 2007). This reflects the higher discriminatory power of the *spa*-typing methodology. Indeed, although MLST-typing distinguishes only major clonal lineages and its discriminatory power is not sufficient for the separation of strains within a clonal group, the method is, nevertheless, useful for describing the evolutionary history of MRSA. *Spa*-typing and MLST-typing are therefore complementary tools.

Overall, 37 different MRSA *spa*-types were identified in this survey, and some of them have not been reported earlier from pigs. In breeding holdings, 15 *spa*-types were found, whereas in production holdings with breeding pigs 34 different *spa*-types were present, including 12 of the 15 *spa*-types identified in breeding holdings and an additional 22 *spa*-types that were not observed in breeding holdings. The higher diversity observed in the production holdings was in line with the greater number of isolates from these holdings.

Most strains isolated from dust samples in the survey were from *spa*-types associated with ST398. This is in line with the findings of the opinion from EFSA's Biological Hazard Panel (EFSA, 2009) that deems that CC398 is the MRSA lineage most often associated with asymptomatic carriage in intensively reared food-producing animals. Among those, *spa*-types t011, t108 and t034 were the predominant types isolated from holdings with breeding pigs in the EU. This is in accordance with data from other studies on MRSA in pigs (de Neeling et al., 2007; Jouy et al., 2009; Tenhagen et al., 2009). MRSA ST398 has also been isolated from food items in the Netherlands (de Boer et al., 2008) and from other farm animal species like dairy cattle (Vicca et al., 2008), veal calves (Graveland et al., 2008), and broilers (Persoons et al., 2009). Moreover, *spa*-types associated with ST398 have also been identified as Methicillin-Sensitive *Staphylococcus aureus* (MSSA) in pig herds in Denmark, Germany and Switzerland (Hasman et al., 2009; Meemken et al., 2009; Riesen und Perreten, 2009).

The multitude of different *spa*-types that was associated with MRSA ST398 in this survey reflects diversity within the ST398. This diversity has also been found in other studies on MRSA ST398 in pigs and in studies on food (Battisti et al., 2009; de Boer et al., 2008; de Neeling et al., 2007; Jouy et al., 2009; Tenhagen et al., 2009).

Surprisingly, some of the *spa*-types that were assigned to ST398 in the survey by MLST did not show *spa* repeat patterns related to t011 or t034 but rather to *spa*-types of ST9 (*spa*-types t899 and t2922). This heterogeneity has also been recently reported for Chinese porcine MRSA isolates (Wagenaar et al., 2009), Italian porcine MRSA isolates (Battisti et al., 2009) as well as Danish bovine and porcine MSSA isolates (Hasman et al., 2009). Likewise, *spa*-type t1939 differed substantially from the most frequent *spa*-types but was nevertheless ST398. An isolate of this *spa*-type has been identified in pigs as MRSA (van Duikeren et al., 2008) and in milk samples from cattle as MSSA (BfR, personal communication). The fact that several isolates harbouring t899 have been associated to ST9 and ST1376 (Hasman et al., 2009; Wagenaar, et al., 2009) highlights the necessity to consider both typing systems complementary.

A minority of the MRSA isolates reported from the survey were not associated with ST398. A total of 41 isolates in the EU and one from Norway (7.5% of all isolates) were from *spa*-types not related to ST398. These were attributed to 13 different *spa*-types that clustered in seven STs. Non-ST398 isolates were detected in breeding holdings in one MS (Italy) and in production holdings in six MSs and one non-MS. However, the majority of non-ST398 isolates were from two MSs, namely Italy and Germany.

The MRSA *spa*-type t127 isolated in the survey belonged to ST1. The *spa*-type has been reported as an MRSA strain being transferred from cattle to humans and vice versa in Hungary (Kaszanyitzky et al., 2007). In addition, this *spa*-type has been isolated as an MSSA from bovine milk samples in Germany (Monecke et al. 2007) and is also a strain known from human infections. Interestingly, the occurrence of this strain was predominantly reported from one MS (Italy, 19 isolates) indicating potential clonal spread within a limited pig population.

The MRSA sequence type ST97, which is linked to *spa*-types 1730, 426, 2112 and 3992 in the survey, has been previously associated with cattle as an MSSA, but has recently also been identified as an MRSA from humans in the United Kingdom. Likewise, it has been isolated as an MRSA from clinical specimens of pigs in Germany (Meemken et al., 2009). This strain was only isolated in a few MSs (Italy: 11 isolates of three *spa*-types, and Germany: three isolates of two *spa*-types) which might also indicate clonal spread in a limited pig population.

MRSA ST9/CC9 found in the survey has recently been reported as the predominant ST in pigs in China (Cui et al., 2009). Isolates of the *spa*-type t1430 related to ST9/CC9 have also been isolated from food items in the Netherlands and Germany (de Boer et al., 2008; Fetsch et al., 2009) and has been identified in MSSA from pigs and cattle in Denmark (Hasman et al., 2009).

The MRSA sequence types ST5, ST8 and ST132 only occurred in individual holdings in this survey and are strains known from human medicine. *Spa*-types t002 (ST5) and t008 (ST8) are frequently reported on the human side. MRSA ST5 t002 is a known strain prevalent in hospitals in central Europe and in France (Karauzum et al., 2008; Krziwanek et al., 2008). In the Ridom-*Spa*-Server-Database (<http://spaserver2.ridom.de>), it accounts for 5.6 % of the recorded MRSA isolates. MRSA ST8 is an internationally known type of MRSA that has been isolated in a number of countries. *Spa*-type t008 accounts for 6.4 % of all isolates in the Ridom-*Spa*-Server-Database. *Spa*-type t1403 (ST132) is less frequently reported to the Ridom *spa*-server database. However, this *spa*-type has been isolated as an MSSA from cattle in Germany (Monecke et al. 2007).

6.5. Importance of the findings on human health

In humans, colonisation with MRSA ST398 originating from pigs has been identified as an occupational health risk for farmers and veterinarians and their families (EFSA, 2009). Although MRSA ST398 represents only a small proportion of the total number of reports of human MRSA infections in the EU, this proportion differs between MSs and in some countries with a low prevalence of human MRSA infection, CC398 is a major contributor to the overall MRSA burden (EFSA, 2009).

In most cases, colonisation with MRSA ST398 in humans is not associated with disease, although clinical cases associated with MRSA ST398 have been reported (van Loo et al., 2007; EFSA, 2009). MRSA ST398 can be introduced into hospitals via colonised farmers and other persons in a region with intensive pig farming. Therefore, MRSA ST398 may add substantially to the MRSA introduced in health care settings (Köck et al., 2009). However, it seems that the capacity for dissemination in humans (patient-to-patient transmission) of livestock-origin MRSA, in particular ST398, is lower as compared to hospital-associated MRSA (Bootsma et al., 2008; Wassenberg et al., 2008).

Also MRSA *spa*-types not belonging to ST398 lineage (MRSA non-ST398) that are already described in humans were detected in this survey from pig holdings. Their presence in the pig population might also pose an occupational risk for humans and a risk of spreading the bacteria to hospital environments.

Food may be contaminated by MRSA (including ST398), however there is currently no evidence for increased risk of human colonisation or infection following contact or consumption of food contaminated by ST398 both in the community and in hospital (EFSA, 2009).

CONCLUSIONS

- This preliminary baseline survey was the first survey on MRSA in holdings with breeding pigs in the EU. It provides comparable estimates of the prevalence of MRSA positive holdings with breeding pigs for the EU MSs and gives an initial picture of the occurrence of MRSA and MRSA ST398 across the EU, even though underestimation of the prevalence has to be considered when interpreting the results. The survey was based on analysing one pooled environmental dust sample collected from each holding.
- This survey has demonstrated that MRSA is commonly detected in holdings with breeding pigs in the EU, even though high disparity between MS situations was observed. Together, 17 MSs out of the participating 24 MSs detected MRSA in their holdings with breeding pigs. MRSA ST398 was by far the most prevalent lineage of MRSA detected in the holdings.
- Considering breeding holdings, considerable disparity in the prevalence of MRSA and of MRSA ST398 was observed among MSs. No breeding holdings tested positive for MRSA in 12 MSs. The EU level prevalence of MRSA and of MRSA ST398 positive breeding holdings were estimated to be 14.0% and 13.1%, respectively. One MS reported MRSA non-ST398 in breeding pig holdings.
- The EU prevalence of MRSA positive production holdings was assessed at 26.9%, whereas the EU prevalence of MRSA ST398 and of MRSA non-ST398 were 25.5% and 1.4%, respectively. However, the variation in prevalence of MRSA and of MRSA ST398 was wide among MSs. No production holdings tested positive in eight MSs. In addition, one non-MS detected MRSA in production holdings.
- Only one MS detected breeding pig holdings positive to MRSA non-ST398, while six MSs and one non-MS reported MRSA non-ST398 in the production pig holdings. The prevalence of MRSA non-ST398 in holdings with breeding pigs across the participating MSs were substantially lower than the prevalence figures obtained for MRSA and MRSA ST398.
- A variety of MRSA *spa*-types were isolated from breeding and production holdings in the EU. However, the t011 *spa*-type was by far the most dominant type found among the isolates in this survey. Also, *spa*-types t108 and t034 were found in relatively high numbers in both breeding and production holdings. The three above-mentioned *spa*-types were associated with ST398/CC398.
- Colonisation of pigs with MRSA ST398 has been identified as an occupational health risk for farmers and veterinarians and their families. In the case of MRSA non-ST398 strains also isolated in this survey from pig holdings, such identification has not yet been made, even though many of the ST types isolated have also been reported from human cases.

RECOMMENDATIONS

- It is recommended that the information from this survey in holdings with breeding pigs be complemented by periodic monitoring of MRSA in breeding and fattening pigs, as well as in other food-producing animal species, such as poultry and cattle, in order to obtain a comprehensive picture of the occurrence of MRSA in these animal populations.
- Investigations on the reasons for the disparity in MRSA prevalence in holdings with breeding pigs among MSs is needed as a basis for the development of potential future intervention measures.
- In MSs with a low or zero MRSA prevalence in this survey, further studies on the emergence of MRSA in pigs could be carried out, since the results from this survey may underestimate the real MRSA prevalence.
- It would be useful to investigate the role of humans as potential sources of MRSA non-ST398, which had not been detected in pigs prior to this survey, as well as the human health importance of the non-ST398 findings in the pig population.

REFERENCES

- Battisti A, Franco A, Merialdi G, Hasman H, Iuresca M, Lorenzetti R, Feltrin F, Zini M, Aarestrup FM, 2009. Heterogeneity among methicillin-resistant *Staphylococcus aureus* from Italian pig finishing holdings. *Veterinary Microbiology* (in press).
- Bootsma MCJ, Trapman P, Wassenberg MWM, Bonten MJM, 2008. A birth-death model with detection to estimate nosocomial transmission rates of two Methicillin-resistant *Staphylococcus aureus* strains. In *Epidemics-1 – First International Conference on Infectious Disease Dynamics*, December 1-3, Asilomar, CA, USA.
- Cui S, Li J, Hu C, Jin S, Li F, Guo Y, Ran L and Ma Y, 2009. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from swine and workers in China 1. *Journal of Antimicrobial Chemotherapy*, 64, pp. 680-683.
- de Boer E, Zwartkruis-Nahuis JT, Wit B, Huijsdens XW, de Neeling AJ, Bosch T, van Oosterom RA, Vila A, Heuvelink AE, 2009. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *International Journal of Food Microbiology*, 134, pp. 52-56.
- de Neeling AJ, van den Broek MJ, Spalburg EC, van Santen-Verheuve MG, Dam-Deisz WD, Boshuizen HC, van de Giessen AW, van Duijkeren E, Huijsdens XW, 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Veterinary Microbiology*, 122, pp. 366-372.
- Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE, 2007. The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clinical Microbiology and Infection*, 13, pp. 222-35.
- EC (European Commission), 2003. Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. OJ L 325, 12.12.2003, p. 31.
- EC (European Commission), 2007a. Commission Decision of 28 September 2007 concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. in herds of breeding pigs to be carried out in the Member States. OJ L 257, 3.10.2007, p. 30.
- EC (European Commission), 2007b. Commission Decision of 20 December 2007 concerning a financial contribution from the Community towards a survey on the prevalence of *Salmonella* spp. and Methicillin-resistant *Staphylococcus aureus* in herds of breeding pigs to be carried out in the Member States. OJ L 14, 17.1.2008, p. 10.
- EFSA, 2007. Report of the Task Force on Zoonoses Data Collection on a proposal for technical specifications for a baseline survey on the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in breeding pigs. *The EFSA Journal*, 129, pp. 1-14.
- EFSA, 2009. Scientific opinion of the panel on Biological Hazards on a request from the European Commission on the assessment of the public health significance of the methicillin resistance of the *Staphylococcus Aureus* (MRSA) in animals and foods. *The EFSA Journal*, 993, pp. 1-73.
- Fetsch A, Tenhagen B-A, Guerra B, Hertwig S, Hammerl JA, Käsbohrer A, Bräunig J, Appel B, 2009. Potential emergence of a non ST398 clone in livestock animals and food, p. P40. In *Third Symposium on Antimicrobial Resistance in Animals and the Environment*, 1-3 June, Tours, France.
- Graveland H, Wagenaar J, Broekhuizen-Stins M, Oosting-Schothorst I, Schoormans A, van Duijkeren E, Huijsdens XW, Mevius D, Heederik D, 2008. Methicillin resistant *Staphylococcus aureus* (MRSA) in veal calf farmers and veal calves in the Netherlands, pp. 62-63. In *National Food Institute (ed.), ASM conference: Antimicrobial resistance in zoonotic bacteria and foodborne pathogens*, Copenhagen, Dk. National Food Institute, Copenhagen, Denmark.

- Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, Vogel U, 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *Journal of Clinical Microbiology*, 41, pp. 5442-8.
- Hasman H, Moodley A, Guardabassi L, Stegger M, Skov R, Aarestrup FM, 2009. *spa*-type distribution in *Staphylococcus aureus* originating from pigs, cattle and poultry. *Veterinary Microbiology* (in press).
- Jouy E, Granier SA, Ruimy R, Felix B, Le Roux A, Pecorella E, Tocqueville V, Kempf I, Sanders P, Andreumont A, Brisabois A, Chauvin C, 2009. Methicillin-resistant *Staphylococcus aureus* (MRSA) in French slaughtered pigs, p. P97. In Third Symposium on Antimicrobial Resistance in Animals and the Environment, 1-3 June, Tours, France.
- Karauzum H, Ferry T, de Bentzmann S, Lina G, Bes M, Vandenesch F, Schmalzer M, Berger-Bachi B, Etienne J, Landmann R, 2008. Comparison of adhesion and virulence of two predominant hospital-acquired methicillin-resistant *Staphylococcus aureus* clones and clonal methicillin-susceptible *S. aureus* isolates 3. *Infection and Immunity*, 76, pp. 5133-5138.
- Kaszanyitzky EJ, Janosi S, Somogyi P, Dan A, van der Graaf-van Bloois L, van Duijkeren E, Wagenaar J, 2007. MRSA transmission between cows and humans. *Emerging Infectious Diseases*, 13, pp. 630-632.
- Köck R, Brakensiek L, Mellmann A, Kipp F, Henderikx M, Harmsen D, Daniels-Haardt I, von Eiff C, Becker K, Hendrix MG, Friedrich AW, 2009. Cross-border comparison of the admission prevalence and clonal structure of methicillin-resistant *Staphylococcus aureus*. *Journal of Hospital Infection*, 71, pp. 320-326.
- Köck R, Harlizius J, Bressan N, Laerberg R, Wieler LH, Witte W, Deurenberg RH, Voss A, Becker K, Friedrich AW, 2009. Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestock-related MRSA into hospitals. *European Journal of Clinical Microbiology and Infectious Diseases*, 28, pp. 1375-1382.
- Krziwanek K, Luger C, Sammer B, Stumvoll S, Stammeler M, Sagel U, Witte W, Mittermayer H, 2008. MRSA in Austria - an overview. *Clinical Microbiology and Infection*, 14, pp. 250-259.
- Meemken D, Blaha T, Tegeler R, Tenhagen B-A, Guerra B, Hammerl JA, Hertwig S, Käsbohrer A, Appel B, Fetsch A, 2009. Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) isolated from lesions of pigs at necropsy in Northwest Germany between 2004 and 2008. *Zoonoses and Public Health* (in press).
- Monecke S, Kuhnert P, Hotzel H, Slickers P and Ehrlich R, 2007. Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. *Veterinary Microbiology*, 15, pp. 128-40.
- Persoons D, Van Hoorebeke S, Hermans K, Butaye P, de Kruif A, Haesebrouck F, Dewulf J, 2009. Methicillin-resistant *Staphylococcus aureus* in poultry. *Emerging Infectious Diseases*, 15, pp. 452-453.
- Riesen A, Perreten V, 2009. Antibiotic resistance and genetic diversity in *Staphylococcus aureus* from slaughter pigs in Switzerland. *Schweiz Arch Tierheilkd.*, 151, pp. 425-431.
- Tenhagen B-A, Fetsch A, Stührenberg B, Schleuter G, Guerra B, Hammerl JA, Hertwig S, Kowall J, Kämpe U, Bräunig J, Schroeter A, Käsbohrer A, Appel B, 2009. Prevalence of MRSA types in slaughter pigs in different German abattoirs. *The Veterinary Record* (in press).
- van Duijkeren E, Ikawaty R, Broekhuizen-Stins J-J, 2008. Transmission of methicillin-resistant *Staphylococcus aureus* between different kinds of pig farms. *Veterinary Microbiology*, 126, pp. 383-389.

- van Loo I, Huijsdens X, Tiemersma E, de Neeling A, Sande-Bruinsma N, Beaujean D, Voss A, Kluytmans J, 2007. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. *Emerging Infectious Diseases*, 13, pp. 1834-1839.
- Vicca J, Vanderhaegehen W, Cerpentier T, Butaye P, 2008. Prevalence at herd level of methicillin resistant *Staphylococcus aureus* in milk samples of dairy herds. In: *Mastitis control from science to practice*. T.J.G.M. Lam (ed.). Wageningen Academic Publishers, Wageningen, pp. 71-75.
- Wagenaar JA, Yue H, Pritchard J, Broekhuizen-Stins M, Huijsdens X, Mevius DJ, Bosch T, van Duijkeren E, 2009. Unexpected sequence types in livestock associated methicillin-resistant *Staphylococcus aureus* (MRSA): MRSA ST9 and a single locus variant of ST9 in pig farming in China 2. *Veterinary Microbiology*. (in press)
- Wassenberg MW, Hopmans TE, Troelstra A, Kluytmans JA, Bonten MJ, 2008. Methicillin-resistant *Staphylococcus aureus* of livestock origin in Dutch hospitals: high-risk patients need only to be investigated if admitted to hospital. *Ned Tijdschr Geneesk*, 152, pp. 2681-8.

APPENDICES

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A. GENERAL FEATURES OF THE EU AND TWO NON-MSs, POPULATION OF HOLDINGS WITH BREEDING PIGS

Table 8: Overview of the population of holdings with breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(a)

Country	Breeding holdings			Production holdings			Total holdings with breeding pigs		
	<50 breeding pigs	≥50 breeding pigs	Total	<50 breeding pigs	≥50 breeding pigs	Total	<50 breeding pigs	≥50 breeding pigs	Total
Austria	128	153	281	11 191	2 703	13 894	11 319	2 856	14 175
Belgium	NA ^(b)	286	286	1	3 731	3 732	1	4 017	4 018
Bulgaria	18	43	61	1	384	385	19	427	446
Cyprus	0	4	4	9	87	96	9	91	100
Czech Republic	26	195	221	988	1 973	2 961	1 014	2 168	3 182
Denmark	12	186	198	852	2 407	3 259	864	2 593	3 457
Estonia	1	7	8	3	28	31	4	35	39
Finland	40	99	139	431	502	933	471	601	1 072
France	12	452	464	2 844	5 746	8 590	2 856	6 198	9 054
Germany	NA	380	380	16 416	12 110	28 526	16 416	12 490	28 906
Hungary	1	39	40	NA	485	485	1	524	525
Ireland	2	39	41	15	290	305	17	329	346
Italy	2 610	186	2 796	5 026	1 018	6 044	7 636	1 204	8 840
Latvia	NA	7	7	NA	39	39	NA	46	46
Lithuania	0	11	11	0	85	85	0	96	96
Luxembourg	0	3	3	14	27	41	14	30	44
Netherlands	29	464	493	185	2 775	2 960	214	3 239	3 453
Poland ^(c)	4 176	1 399	5 575	159 101	3 926	163 027	163 277	5 265	168 542
Portugal	NA	39	39	2 804	836	3 640	2 804	875	3 679
Slovakia	41	93	134	1 088	207	1 295	1 129	300	1 429
Slovenia	12	21	33	5 852	49	5 901	5 864	70	5 934
Spain	39	415	454	21 865	12 449	34 314	21 904	12 864	34 768
Sweden	3	76	79	596	761	1 357	599	837	1 436
United Kingdom	NA	130	130	NA	1 539	1 539	NA	1 669	1 669
EU (24 MSs)	7 150	4 727	11 877	229 282	54 157	283 439	236 432	58 824	295 256
Norway	11	124	135	903	631	1 534	914	755	1 669
Switzerland	27	87	114	1 149	821	1 970	1 176	908	2 084

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

^(b): NA = data not reported

^(c): 2009 data

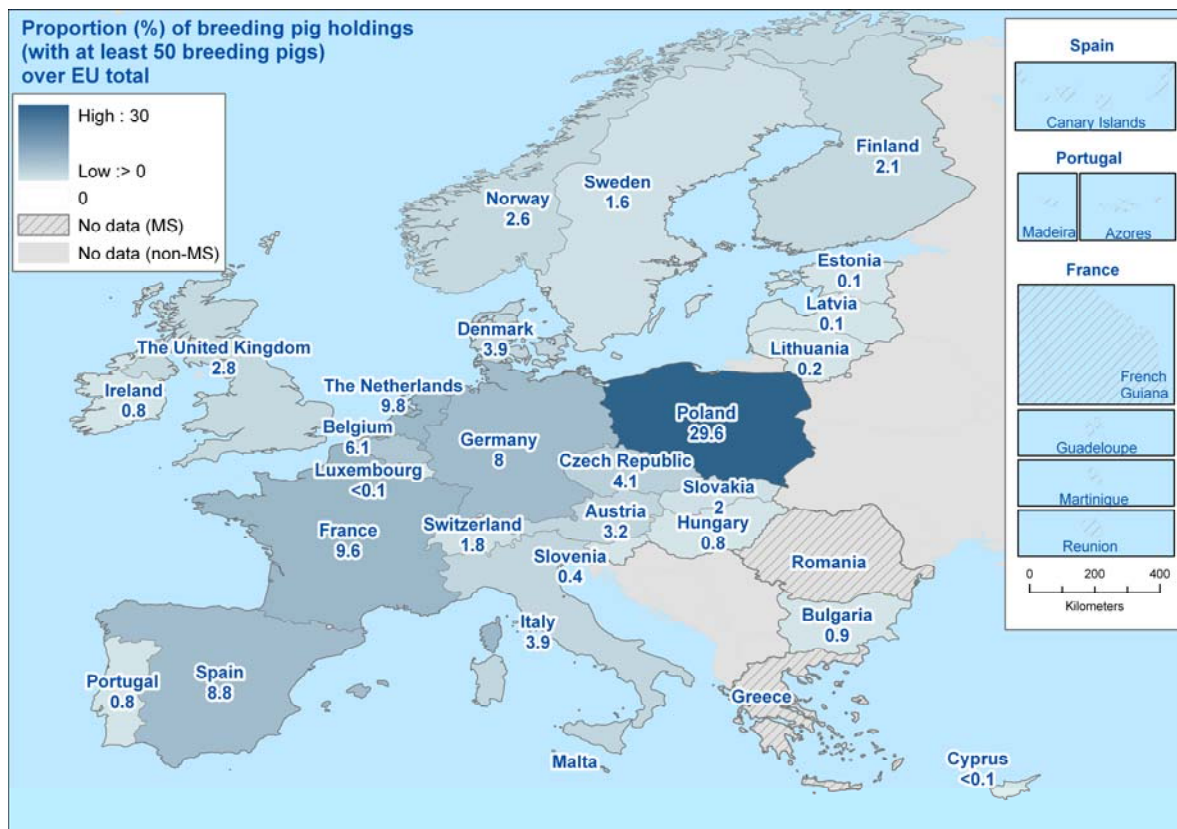


Figure 13: Proportion (%) of breeding holdings with at least 50 breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(a)

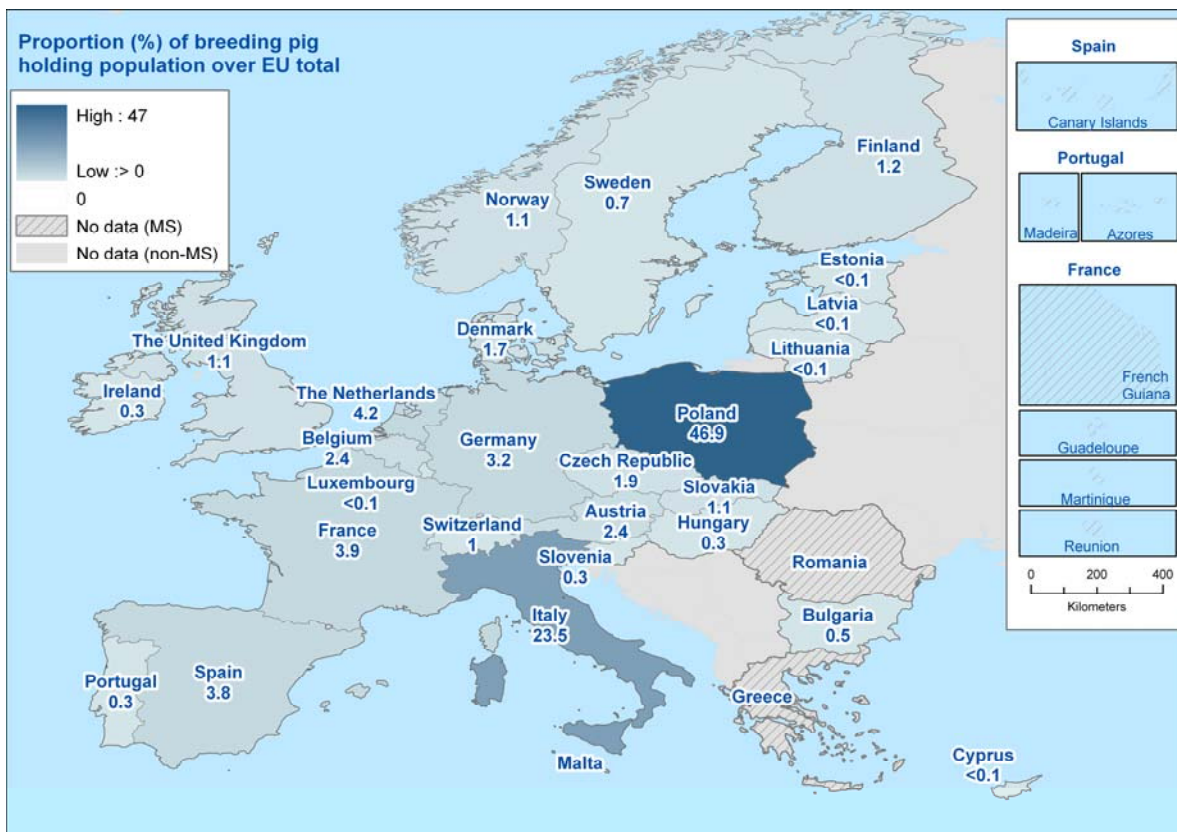


Figure 14: Proportion (%) of breeding holdings population, MRSA EU baseline survey in breeding pigs, 2008^(a)

(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

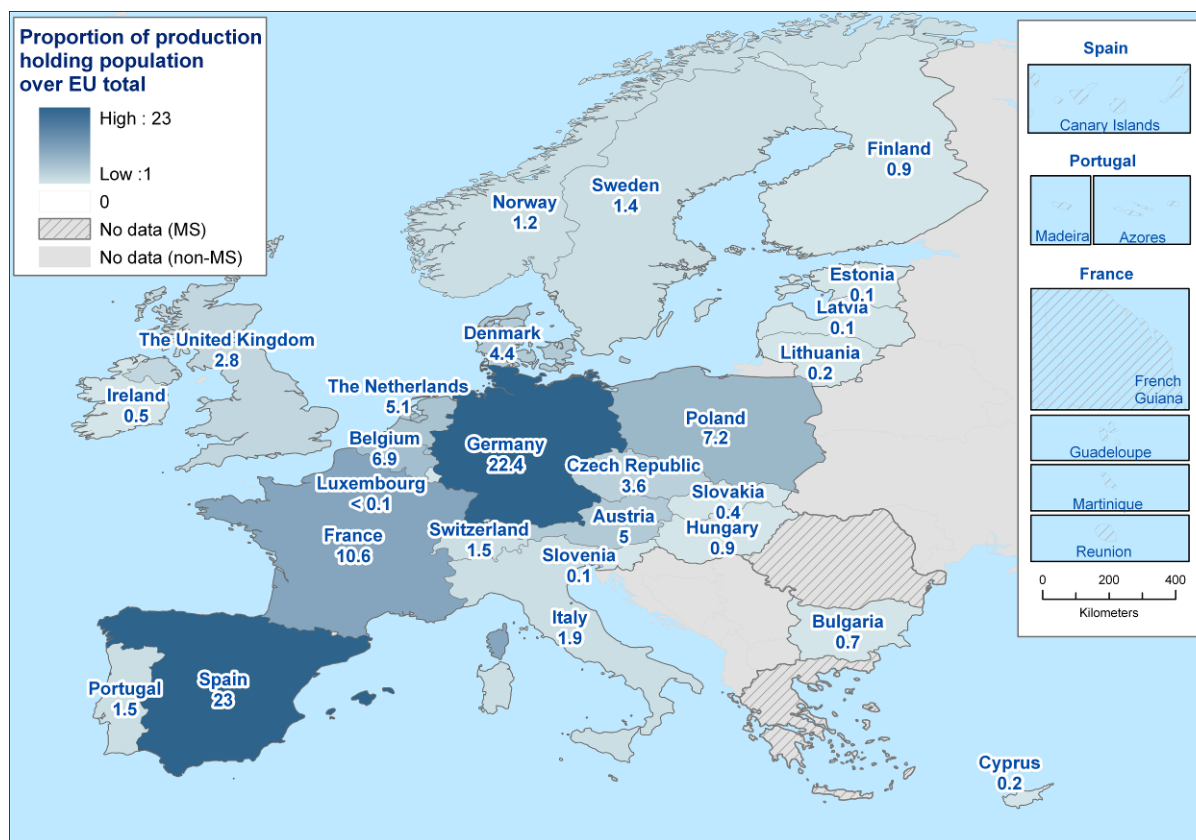


Figure 15: Proportion (%) of production pig holdings with at least 50 breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(a)

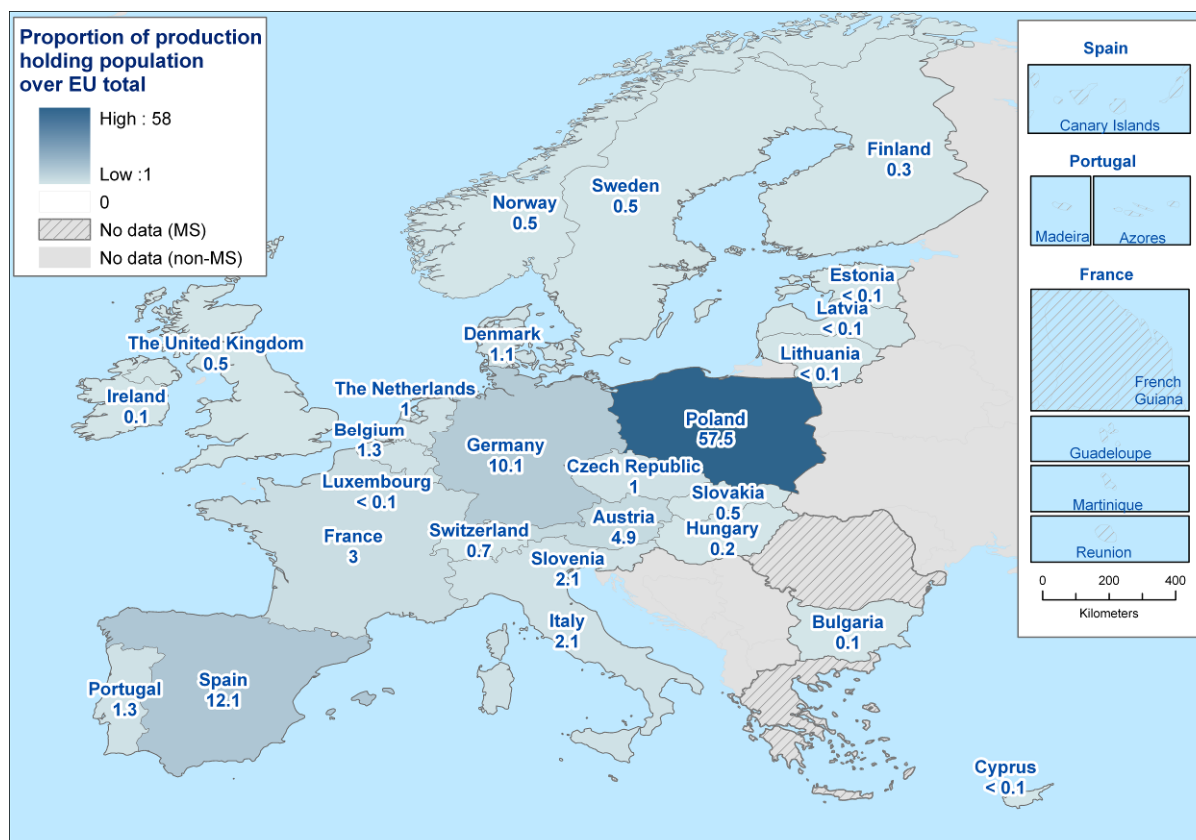


Figure 16: Proportion (%) of production holdings with breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(a)

(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

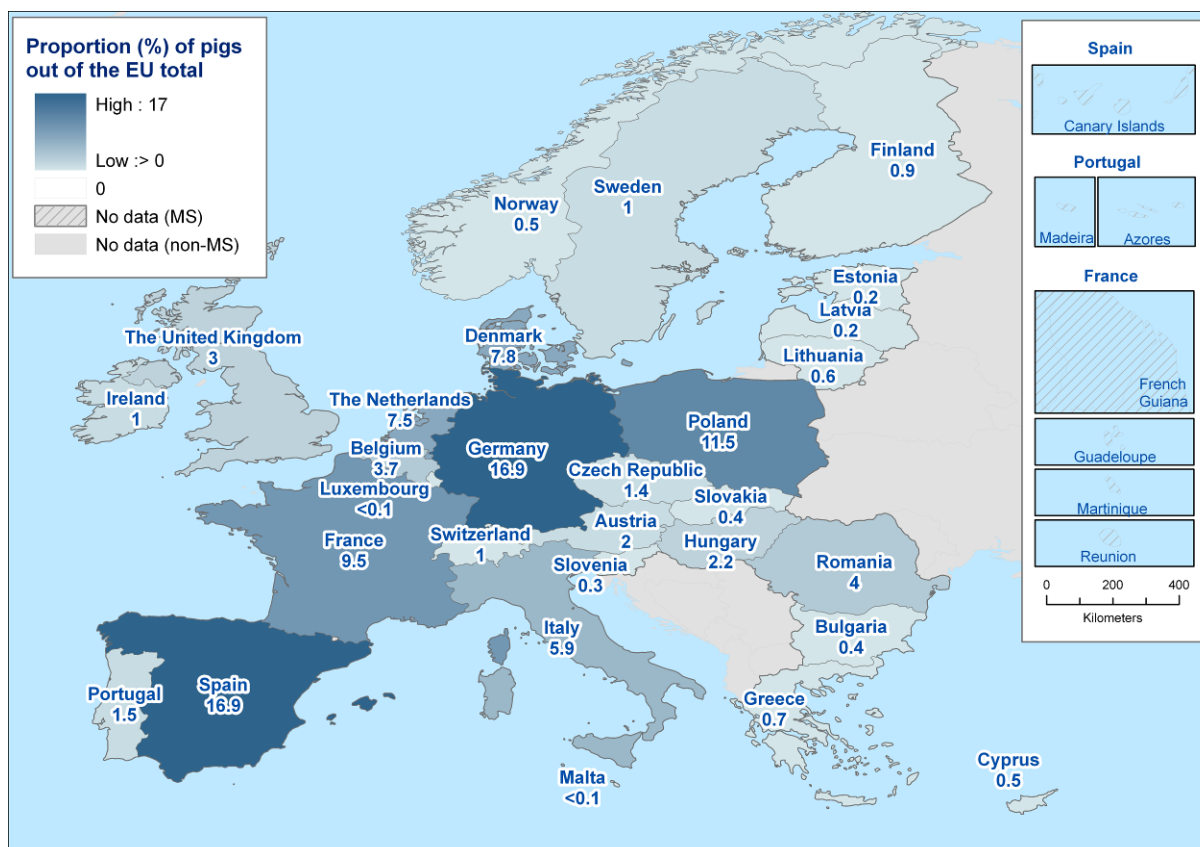


Figure 17: Proportion (%) of pig population, MRSA EU baseline survey in breeding pigs, 2008^(a)

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

B. LIST OF CRITERIA USED TO IDENTIFY NON-VALID AND NON-PLAUSIBLE INFORMATION IN THE MRSA DATABASE, MRSA BASELINE SURVEY IN THE EU, 2008

The variables are uniquely identified using the 'item integer' mentioned in the ad hoc Data Dictionary.

Criterion	Rationale for the criterion
C1	003 Date of sampling: >15 January 2009 This criterion excludes all records containing a date of sampling after 15 January 2009.
C2	063 Date of bacteriological detection testing: < 15 December 2007 This criterion excludes all records containing a date of primary testing in the laboratory before 15 December 2007.
C3	063 Date of bacteriological detection testing: < value of 003 Date of sampling This criterion excludes all records containing a date of primary testing in the laboratory before the date of sampling.
C4	Difference between '003 Date of sampling' and '063 Date of bacteriological detection testing': >13 This criterion excludes all records containing a 'days to bacteriological start of test' over 13 days.
C5	066 <i>Spa</i>-typing: 'is null (empty)' and 064 MRSA confirmation is 'confirmed' This criterion excludes all records containing positive test results with no information on <i>Spa</i> -typing
C6	066 <i>Spa</i> typing: 'is not null (not empty)' and 064 MRSA confirmation is 'not confirmed' This criterion excludes all records containing negative test results with information on <i>Spa</i> -typing.

C. STATISTICAL METHODOLOGY USED IN THE ANALYSIS OF THE BASELINE SURVEY ON THE PREVALENCE OF MRSA IN HERDS WITH BREEDING PIGS IN THE EU, 2008

Observed prevalence in each participating country

The observed prevalence rate of MRSA in each country was calculated as the fraction of MRSA positive holdings in this country, including a 95% CI based on a finite population approach. The finite population approach is appropriate in the case when there are only a limited number of holdings (N) in a country, of which a considerable part (n) has been sampled. The approach assumes that the prevalence in the n holdings that were sampled is certain. Therefore, if a country has only five holdings, and they are all in the sample (census sampling), the prevalence in this country, according to the finite population approach, is known with complete certainty. One caveat, however, is that holdings were sampled only at a single moment in time, and that the persistence of MRSA positivity in time is uncertain. It might well be that a holding that is found positive in one part of the year might be negative in another. Without knowledge of the magnitude of the within-holding correlation of positive findings, however, this cannot be taken into account. This is especially influential in the case of census sampling, where the finite population approach gives a CI that is equal to the point estimate. The true CI is likely to be larger, but cannot be calculated.

In order to estimate a CI based on a finite population, the total number of holdings in each country (N) must be known. We used the number of breeding pig/production pig holdings with a least 50 breeding pigs for all countries that exclusively sampled breeding pig/production pig holdings with at least 50 breeding pigs. Also, in the case that holdings with less than 50 breeding pigs were sampled, we used the total number of breeding pigs in the country. This choice does not influence the point estimate, only the calculation of the CI. Exact 95% CIs were calculated based on the hypergeometric distribution.

Prevalence at EU level

The prevalence estimate at EU level was calculated by weighting each MS prevalence with the fractions of all EU holdings that are found in the MS. As the survey aims to estimate the prevalence in holdings with at least 50 breeding pigs (covering at least 80% of the total breeding pig population), we estimated the EU level prevalence p_{EU} based on the data from holdings with at least 50 breeding pigs, using the following formula:

$$p_{EU} = \sum_{MS} \frac{\#holdings_{MS}}{\sum_{MS} \#holdings_{MS}} p_{MS} \quad (1) \Leftrightarrow p_{EU} = \frac{\sum_{MS} (1/f_{MS}) (\# \text{ positive holdings in sample}_{MS})}{\sum_{MS} \#holdings} \quad (2)$$

Here p_{MS} is the prevalence rate of MRSA positive holdings in holdings with at least 50 breeding pigs in the MS and $\#holdings_{MS}$ is the total number of holdings with at least 50 breeding pigs in the MS. f_{MS} is the sampling fraction of holdings with at least 50 breeding pigs in the MS. These numbers were provided by MSs (Appendix A). Note that while holdings with less than 50 breeding pigs were not included in this estimate, they were included in the estimates for the individual MS. In these individual MSs these holdings are needed to make the survey cover at least 80% of the breeding pigs in the MS, while this is not the case at EU level.

An alternative would have been to use $\#holdings_{MS}$ equal to the total number of holdings in the MS, and include all sampled holdings. However, in many MSs, this would mean extrapolating findings from holdings with at least 50 breeding pigs to a large number of smaller holdings, while no such holdings are in the sample. As smaller holdings might have a different chance of being positive for MRSA, such an extrapolation cannot be justified. The EU-prevalence was estimated using SAS 9.2, PROC SURVEYREG, including only holdings with at least 50 breeding pigs, both in case of the sample of holdings, and for the total number of holdings in each MS.

D. OVERVIEW OF THE NUMBER OF HOLDINGS WITH AT LEAST 50 BREEDING PIGS, MRSA EU BASELINE SURVEY, 2008^(a)

Table 9: Number of holdings with at least 50 breeding pigs, sampled in participating countries, sampling fractions and related weights, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	Breeding holdings with at least 50 breeding pigs				Production holdings with at least 50 breeding pigs			
	No of Sampled Holdings	Total No of Holdings	Samplin g Fraction	Weight	No of Sampled Holdings	Total No of Holdings	Samplin g Fraction	Weight
Austria	61	153	0.399	2.51	129	2,703	0.048	20.95
Belgium	15	286	0.052	19.07	179	3,731	0.048	20.84
Bulgaria	43	43	1.000	1.00	24	384	0.063	16.00
Cyprus	4	4	1.000	1.00	65	87	0.747	1.34
Czech Republic	106	195	0.544	1.84	160	1,973	0.081	12.33
Denmark	95	186	0.511	1.96	195	2,407	0.081	12.34
Estonia	6	7	0.857	1.17	28	28	1.000	1.00
Finland	48	99	0.485	2.06	142	502	0.283	3.54
France	156	452	0.345	2.90	184	5,746	0.032	31.23
Germany	46	380	0.121	8.26	155	12,110	0.013	78.13
Hungary	39	39	1.000	1.00	141	485	0.291	3.44
Ireland	38	39	0.974	1.03	148	290	0.510	1.96
Italy	43	186	0.231	4.33	169	1,018	0.166	6.02
Latvia	5	7	0.714	1.40	28	39	0.718	1.39
Lithuania	10	11	0.909	1.10	72	85	0.847	1.18
Luxembourg	3	3	1.000	1.00	27	27	1.000	1.00
Netherlands	109	464	0.235	4.26	212	2,775	0.076	13.09
Poland	120	2,094	0.057	17.45	157	3,926	0.040	25.01
Portugal	34	39	0.872	1.15	133	836	0.159	6.29
Slovakia	93	93	1.000	1.00	86	207	0.415	2.41
Slovenia	23	21	1.095	1 ^(b)	47	49	0.959	1.04
Spain	150	415	0.361	2.77	209	12,449	0.017	59.56
Sweden	54	76	0.711	1.41	131	761	0.172	5.81
United Kingdom	67	130	0.515	1.94	191	1,539	0.124	8.06
Norway	82	124	0.661	1.51	74	631	0.117	8.53
Switzerland	71	87	0.816	1.23	154	821	0.188	5.33

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

^(b): As the number of breeding holdings in the sample was larger than the number of breeding holdings reported by the country, the sampling fraction was assumed to be 1. The discrepancy appears because the “number of holdings with at least 50 breeding pigs” was reported on the basis of the number of breeding pigs on the holdings in December 2007 and the “number of sampled holdings with at least 50 breeding pigs” was calculated on the basis of number of breeding pigs reported during the sampling in 2008.

E. RESULTS OF THE DESCRIPTIVE ANALYSIS OF THE SAMPLE DATA OF THE MRSA BASELINE SURVEY IN HOLDINGS WITH BREEDING PIGS^(a)

Table 10: Number and percentage of breeding holdings included in the survey by the month of sampling, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	Month of sampling												Total
	Jan 2008	Feb 2008	Mar 2008	Apr 2008	May 2008	Jun 2008	Jul 2008	Aug 2008	Sep 2008	Oct 2008	Nov 2008	Dec 2008	
Austria	7(9.3%)	5(6.7%)	6(8.0%)	6(8.0%)	8(10.7%)	7(9.3%)	34.0%)	9(12.0%)	2(2.7%)	6(8.0%)	8(10.7%)	8(10.7%)	75
Belgium			1(6.7%)	3(20.0%)	1(6.7%)		1(6.7%)	2(13.3%)		2(13.3%)	3(20.0%)	2(13.3%)	15
Bulgaria			1(2.1%)		1(2.1%)	7(14.9%)	1(2.1%)			17(36.2%)	17(36.2%)	3(6.4%)	47
Cyprus	1(25.0%)			1(25.0%)			1(25.0%)			1(25.0%)			4
Czech Republic		5(4.7%)	19(17.9%)	16(15.1%)	16(15.1%)	11(10.4%)	9(8.5%)	7(6.6%)	6(5.7%)	9(8.5%)	8(7.5%)		106
Denmark		1(1.1%)	5(5.3%)	5(5.3%)	9(9.5%)	13(13.7%)	8(8.4%)	7(7.4%)	16(16.8%)	10(10.5%)	13(13.7%)	8(8.4%)	95
Estonia	1(16.7%)			2(33.3%)	1(16.7%)	1(16.7%)						1(16.7%)	6
Finland	3(6.3%)	4(8.3%)	4(8.3%)	2(4.2%)	4(8.3%)	4(8.3%)	3(6.3%)	5(10.4%)	5(10.4%)	2(4.2%)	5(10.4%)	7(14.6%)	48
France	4(2.5%)	17(10.8%)	13(8.3%)	14(8.9%)	16(10.2%)	19(12.1%)	12(7.6%)	12(7.6%)	16(10.2%)	11(7.0%)	16(10.2%)	7(4.5%)	157
Germany		1(2.2%)	4(8.7%)	3(6.5%)	2(4.3%)	5(10.9%)	3(6.5%)	5(10.9%)	8(17.4%)	5(10.9%)	4(8.7%)	6(13.0%)	46
Hungary			1(2.5%)	6(15.0%)	8(20.0%)	3(7.5%)	4(10.0%)	2(5.0%)	2(5.0%)	5(12.5%)	2(5.0%)	7(17.5%)	40
Ireland	2(5.0%)	3(7.5%)	1(2.5%)	4(10.0%)	1(2.5%)	6(15.0%)	5(12.5%)	2(5.0%)	6(15.0%)	6(15.0%)	2(5.0%)	2(5.0%)	40
Italy			1(2.3%)	4(9.3%)	3(7.0%)	6(14.0%)	4(9.3%)	4(9.3%)	6(14.0%)	5(11.6%)	8(18.6%)	2(4.7%)	43
Latvia						1(20.0%)	1(20.0%)	1(20.0%)	2(40.0%)				5
Lithuania			4(40.0%)					1(10.0%)	1(10.0%)	1(10.0%)	1(10.0%)	2(20.0%)	10
Luxembourg				1(33.3%)							2(66.7%)		3
Poland	10(7.0%)	13(9.1%)	16(11.2%)	11(7.7%)	8(5.6%)	15(10.5%)	13(9.1%)	6(4.2%)	13(9.1%)	15(10.5%)	12(8.4%)	11(7.7%)	143
Portugal											8(23.5%)	26(76.5%)	34
Slovakia		18(18.8%)	22(22.9%)	14(14.6%)	13(13.5%)	5(5.2%)	2(2.1%)	6(6.3%)	3(3.1%)	3(3.1%)	6(6.3%)	4(4.2%)	96
Slovenia	1(3.7%)	14(51.9%)	3(11.1%)	2(7.4%)	3(11.1%)			1(3.7%)	1(3.7%)	1(3.7%)	1(3.7%)		27
Spain	6(4.0%)	12(8.0%)	7(4.7%)	14(9.3%)	12(8.0%)	20(13.3%)	15(10.0%)	6(4.0%)	7(4.7%)	16(10.7%)	22(14.7%)	13(8.7%)	150
Sweden	5(9.1%)	3(5.5%)	2(3.6%)	8(14.5%)	5(9.1%)	4(7.3%)	5(9.1%)	4(7.3%)	5(9.1%)	6(10.9%)	4(7.3%)	4(7.3%)	55
Netherlands	4(3.7%)	1(0.9%)	3(2.8%)	8(7.3%)	8(7.3%)	12(11.0%)	14(12.8%)	8(7.3%)	19(17.4%)	14(12.8%)	18(16.5%)		109
United Kingdom	1(1.5%)	6(9.0%)	8(11.9%)	2(3.0%)	7(10.4%)	6(9.0%)	8(11.9%)	4(6.0%)	5(7.5%)	7(10.4%)	6(9.0%)	7(10.4%)	67
European Union	45(3.2%)	103(7.2%)	121(8.5%)	126(8.9%)	126(8.9%)	145(10.2%)	112(7.9%)	92(6.5%)	123(8.7%)	142(10.0%)	166(11.7%)	120(8.4%)	1 421
Norway		6(5.6%)	8(7.4%)	20(18.5%)	15(13.9%)	5(4.6%)	1(0.9%)	10(9.3%)	11(10.2%)	15(13.9%)	5(4.6%)	12(11.1%)	108
Switzerland	7(9.9%)	7(9.9%)	6(8.5%)	2(2.8%)	7(9.9%)	5(7.0%)	8(11.3%)	7(9.9%)	6(8.5%)	6(8.5%)	8(11.3%)	2(2.8%)	71

(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

Table 11: Number and percentage of production holdings with breeding pigs included in the survey by the month of sampling, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	Month of sampling												Total
	Jan 2008	Feb 2008	Mar 2008	Apr 2008	May 2008	Jun 2008	Jul 2008	Aug 2008	Sep 2008	Oct 2008	Nov 2008	Dec 2008	
Austria	11 (6.6%)	18 (10.8%)	16 (9.6%)	13 (7.8%)	6 (3.6%)	23 (13.8%)	12 (7.2%)	12 (7.2%)	16 (9.6%)	9 (5.4%)	11 (6.6%)	20 (12.0%)	167
Belgium			14 (7.6%)	9 (4.9%)	19 (10.3%)	20 (10.9%)	9 (4.9%)	13 (7.1%)	17 (9.2%)	33 (17.9%)	29 (15.8%)	21 (11.4%)	184
Bulgaria	6 (9.2%)	2 (8.0%)	2 (8.0%)		1 (4.0%)	5 (20.0%)				9 (36.0%)	4 (16.0%)	2 (8.0%)	25
Cyprus		5 (7.7%)	6 (9.2%)	5 (7.7%)	5 (7.7%)	5 (7.7%)	6 (9.2%)	5 (7.7%)	6 (9.2%)	3 (4.6%)	6 (9.2%)	7 (10.8%)	65
Czech Republic		10 (6.2%)	15 (9.3%)	30 (18.6%)	20 (12.4%)	15 (9.3%)	19 (11.8%)	15 (9.3%)	18 (11.2%)	7 (4.3%)	12 (7.5%)		161
Denmark	1 (0.5%)	6 (3.0%)	13 (6.6%)	14 (7.1%)	14 (7.1%)	17 (8.6%)	7 (3.5%)	10 (5.1%)	20 (10.1%)	29 (14.6%)	33 (16.7%)	34 (17.2%)	198
Estonia	2 (7.1%)	3 (10.7%)	3 (10.7%)	1 (3.6%)	2 (7.1%)	2 (7.1%)	3 (10.7%)	2 (7.1%)	4 (14.3%)	2 (7.1%)	3 (10.7%)	1 (3.6%)	28
Finland	7 (4.7%)	13 (8.7%)	4 (2.7%)	15 (10.0%)	6 (4.0%)	16 (10.7%)	13 (8.7%)	11 (7.3%)	14 (9.3%)	20 (13.3%)	9 (6.0%)	22 (14.7%)	150
France	6 (3.2%)	15 (8.1%)	18 (9.7%)	19 (10.3%)	15 (8.1%)	16 (8.6%)	22 (11.9%)	14 (7.6%)	18 (9.7%)	18 (9.7%)	15 (8.1%)	9 (4.9%)	185
Germany	3 (1.9%)	1 (0.6%)	12 (7.7%)	6 (3.9%)	3 (1.9%)	20 (12.9%)	10 (6.5%)	6 (3.9%)	23 (14.8%)	31 (20.0%)	24 (15.5%)	16 (10.3%)	155
Hungary		1 (0.7%)	15 (10.6%)	12 (8.5%)	12 (8.5%)	12 (8.5%)	8 (5.7%)	13 (9.2%)	18 (12.8%)	10 (7.1%)	7 (5.0%)	33 (23.4%)	141
Ireland	8 (5.4%)	9 (6.0%)	8 (5.4%)	14 (9.4%)	13 (8.7%)	10 (6.7%)	7 (4.7%)	8 (5.4%)	11 (7.4%)	17 (11.4%)	35 (23.5%)	9 (6.0%)	149
Italy			4 (2.3%)	5 (2.9%)	11 (6.4%)	24 (14.0%)	9 (5.3%)	15 (8.8%)	24 (14.0%)	30 (17.5%)	32 (18.7%)	17 (9.9%)	171
Latvia					4 (14.3%)	7 (25.0%)	3 (10.7%)	3 (10.7%)	4 (14.3%)	2 (7.1%)	4 (14.3%)	1 (3.6%)	28
Lithuania			25 (34.7%)	3 (4.2%)	5 (6.9%)	3 (4.2%)	6 (8.3%)	3 (4.2%)	6 (8.3%)	7 (9.7%)	8 (11.1%)	6 (8.3%)	72
Luxembourg	3 (7.3%)	3 (7.3%)	3 (7.3%)	3 (7.3%)	3 (7.3%)	4 (9.8%)	4 (9.8%)	4 (9.8%)	5 (12.2%)	4 (9.8%)	3 (7.3%)	2 (4.9%)	41
Poland	10 (5.6%)	20 (11.2%)	10 (5.6%)	16 (9.0%)	12 (6.7%)	15 (8.4%)	14 (7.9%)	11 (6.2%)	13 (7.3%)	21 (11.8%)	12 (6.7%)	24 (13.5%)	178
Portugal											18 (13.2%)	118 (86.8%)	136
Slovakia		3 (3.1%)	2 (2.1%)	1 (1.0%)	2 (2.1%)	16 (16.7%)	15 (15.6%)	11 (11.5%)	11 (11.5%)	12 (12.5%)	10 (10.4%)	13 (13.5%)	96
Slovenia	2 (2.3%)	10 (11.5%)	12 (13.8%)	9 (10.3%)	12 (13.8%)	8 (9.2%)	6 (6.9%)	4 (4.6%)	4 (4.6%)	9 (10.3%)	9 (10.3%)	2 (2.3%)	87
Spain	5 (2.4%)	28 (13.4%)	11 (5.3%)	19 (9.1%)	23 (11.0%)	13 (6.2%)	22 (10.5%)	17 (8.1%)	27 (12.9%)	25 (12.0%)	12 (5.7%)	7 (3.3%)	209
Sweden	12 (8.2%)	16 (10.9%)	15 (10.2%)	5 (3.4%)	13 (8.8%)	15 (10.2%)	9 (6.1%)	13 (8.8%)	12 (8.2%)	12 (8.2%)	11 (7.5%)	14 (9.5%)	147
Netherlands	5 (2.4%)	6 (2.8%)	6 (2.8%)	15 (7.1%)	26 (12.3%)	14 (6.6%)	19 (9.0%)	19 (9.0%)	21 (9.9%)	37 (17.5%)	41 (19.3%)	3 (1.4%)	212
United Kingdom	6 (3.1%)	7 (3.7%)	9 (4.7%)	9 (4.7%)	12 (6.3%)	12 (6.3%)	17 (8.9%)	16 (8.4%)	18 (9.4%)	25 (13.1%)	36 (18.8%)	24 (12.6%)	191
European Union	87 (2.7%)	176 (5.5%)	223 (7.0%)	223 (7.0%)	239 (7.5%)	292 (9.2%)	240 (7.6%)	225 (7.1%)	310 (9.8%)	372 (11.7%)	384 (12.1%)	405 (12.8%)	3 176
Norway		22 (15.4%)	11 (7.7%)	24 (16.8%)	9 (6.3%)	10 (7.0%)	2 (1.4%)	12 (8.4%)	20 (14.0%)	12 (8.4%)	14 (9.8%)	7 (4.9%)	143
Switzerland	4 (2.6%)	14 (9.1%)	11 (7.1%)	15 (9.7%)	13 (8.4%)	16 (10.4%)	17 (11.0%)	13 (8.4%)	15 (9.7%)	16 (10.4%)	13 (8.4%)	7 (4.5%)	154

(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

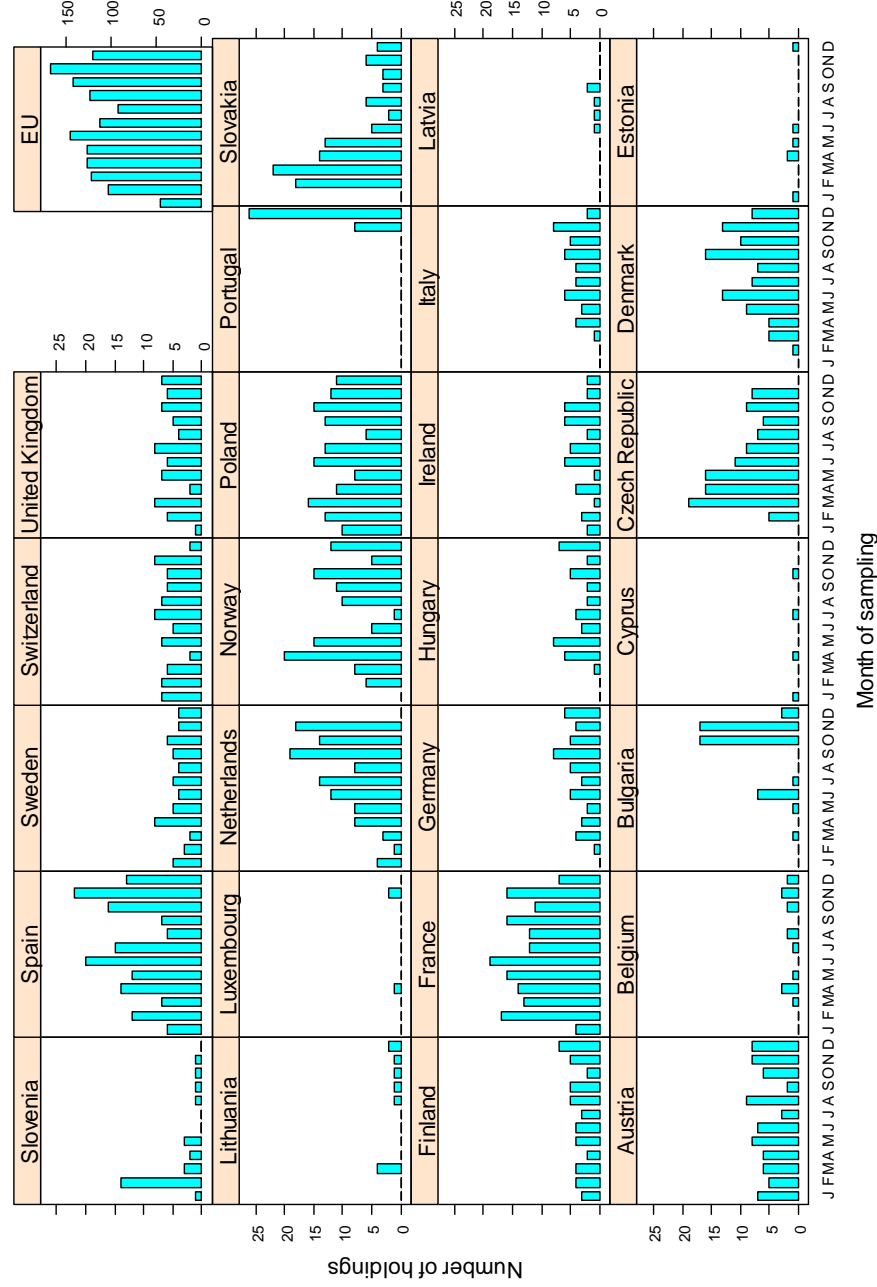


Figure 18: Distribution of the number of sampled breeding holdings by month of sampling^(a), MRSA EU baseline survey in breeding pigs, 2008^(b)

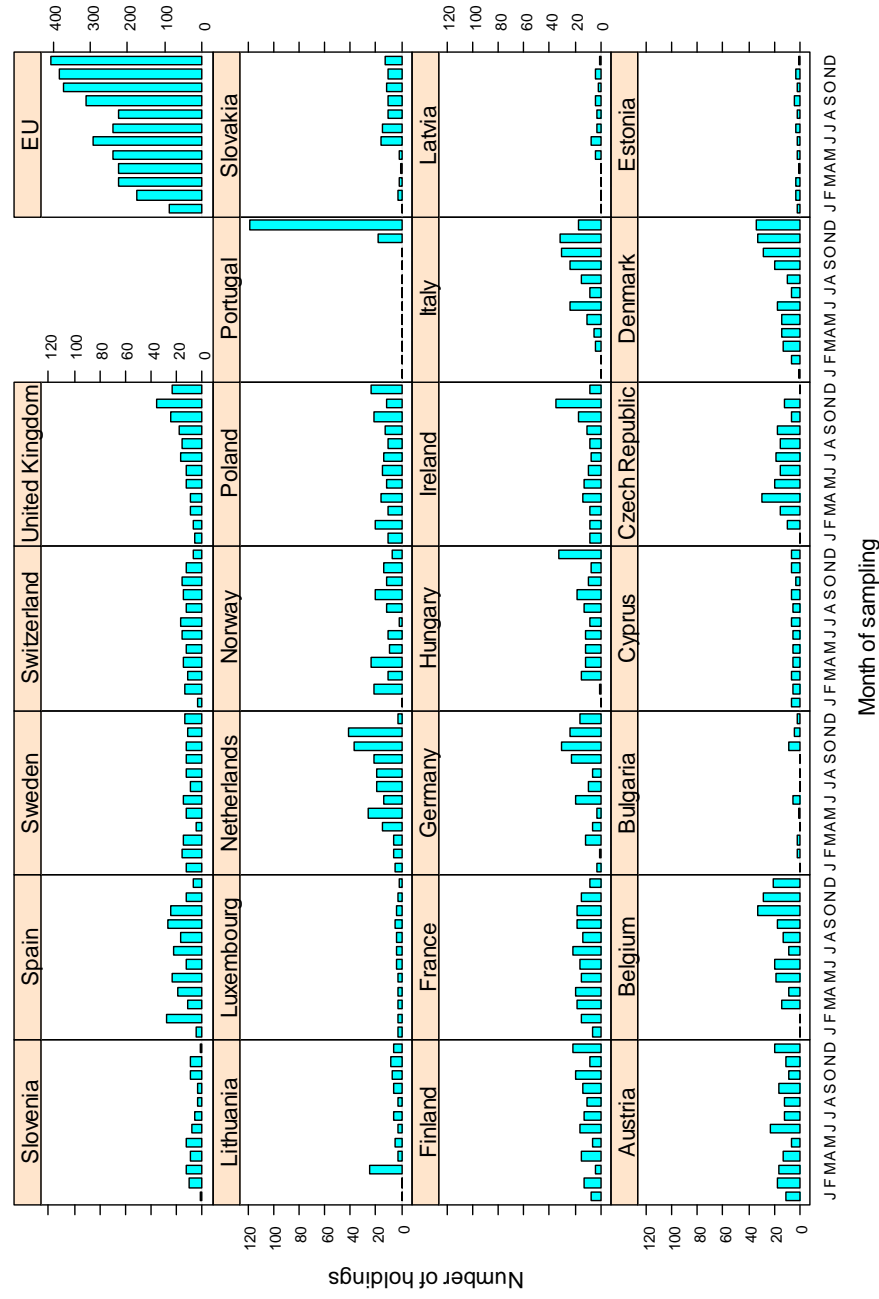


Figure 19: Distribution of the number of sampled production holdings with breeding pigs by month of sampling^(a), MRSA EU baseline survey in breeding pigs, 2008^(b)

- (a): Holdings collected from 1 December to 15 January have been considered together in these graphs (D, in the x axis).
 (b): Greece, Malta and Romania did not conduct the survey and two non-MISs, Norway and Switzerland, participated.

Table 12: Distribution of the number of sampled breeding holdings by the size of holding, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	Size of the holding										Total
	<50		50-99		100-399		400-999		>999		
	N	%	N	%	N	%	N	%	N	%	
Austria	14	18	34	45	24	32	2	2	1	1	75
Belgium			2	13	13	86					15
Bulgaria	4	8	7	14	10	21	5	10	21	44	47
Cyprus					1	25	1	25	2	50	4
Czech Republic			8	7	26	24	26	24	46	43	106
Denmark			1	1	39	41	49	51	6	6	95
Estonia					5	83	1	16			6
Finland			20	41	23	47	4	8	1	2	48
France	1	0	20	12	121	77	15	9			157
Germany			3	6	23	50	13	28	7	15	46
Hungary	1	2	3	7	14	35	14	35	8	20	40
Ireland	2	5	8	20	15	37	14	35	1	2	40
Italy			2	4	18	41	14	32	9	20	43
Latvia					2	40	1	20	2	40	5
Lithuania					5	50	3	30	2	20	10
Luxembourg			1	33	1	33	1	33			3
Netherlands			6	5	73	66	24	22	6	5	109
Poland	23	16	26	18	65	45	18	12	11	7	143
Portugal					15	44	18	52	1	2	34
Slovakia	3	3	19	19	58	60	13	13	3	3	96
Slovenia	4	14	16	59	7	25					27
Spain			11	7	54	36	47	31	38	25	150
Sweden	1	1	10	18	41	74	3	5			55
United Kingdom			4	5	20	29	36	53	7	10	67
European Union	53	3	201	14	673	47	322	22	172	12	1 421
Norway	26	24	48	44	33	30	1	0			108
Switzerland			45	63	26	36					71

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

Table 13: Distribution of the number of sampled production holding with breeding pigs by the size of the holding, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	Size of the holding										Total
	<50		50-99		100-399		400-999		>999		
	N	%	N	%	N	%	N	%	N	%	
Austria	38	22	101	60	27	16	1	0			167
Belgium	5	2	47	25	123	66	8	4	1	0	184
Bulgaria	1	4	7	28	4	16	5	20	8	32	25
Cyprus			2	3	30	46	24	37	9	14	65
Czech Republic	1	0	1	0	23	14	35	21	101	62	161
Denmark	3	1	11	5	79	39	84	42	21	10	198
Estonia					14	50	12	42	2	7	28
Finland	8	5	65	43	63	42	9	6	5	3	150
France	1	0	41	22	129	69	11	5	3	1	185
Germany			22	14	103	66	15	9	15	9	155
Hungary					20	14	47	33	74	52	141
Ireland	1	0	20	13	75	50	44	29	9	6	149
Italy	2	1	21	12	76	44	51	29	21	12	171
Latvia					14	50	5	17	9	32	28
Lithuania					21	29	18	25	33	45	72
Luxembourg	14	34	13	31	12	29	2	4			41
Netherlands			4	1	104	49	88	41	16	7	212
Poland	21	11	30	16	81	45	23	12	23	12	178
Portugal	3	2	25	18	92	67	15	11	1	0	136
Slovakia	10	10	31	32	42	43	10	10	3	3	96
Slovenia	40	45	35	40	5	5	1	1	6	6	87
Spain			35	16	72	34	56	26	46	22	209
Sweden	16	10	49	33	61	41	16	10	5	3	147
United Kingdom			21	10	83	43	71	37	16	8	191
European Union	164	5	581	18	1 353	43	651	20	427	13	3 176
Norway	69	48	56	39	15	10	3	2			143
Switzerland			101	65	51	33	2	1			154

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

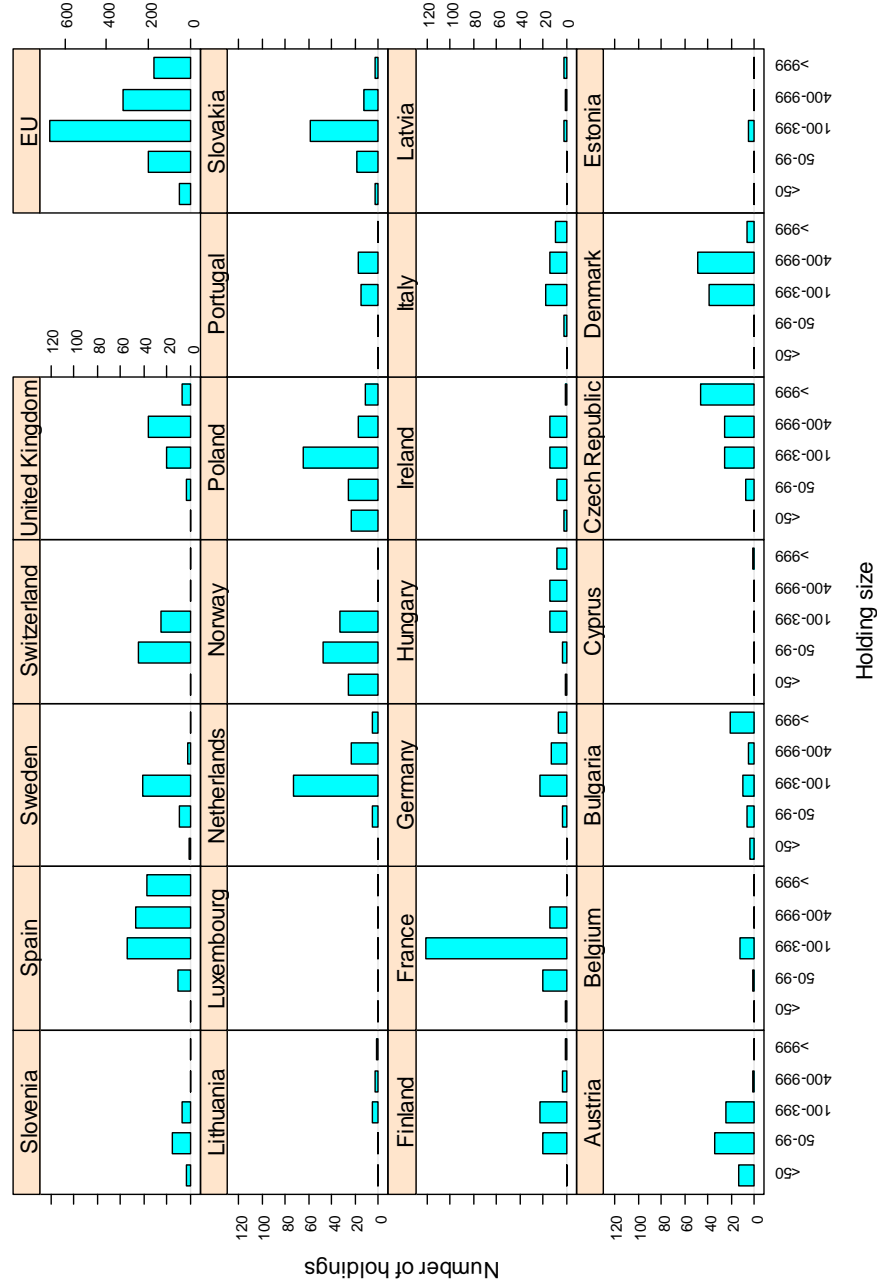


Figure 20: Distribution of the number of sampled breeding holdings by the size of the holding, MRSA EU baseline survey in breeding pigs, 2008^(a)

(a): Greece, Malta and Romania did not conduct the survey and two non-Ms, Norway and Switzerland, participated.

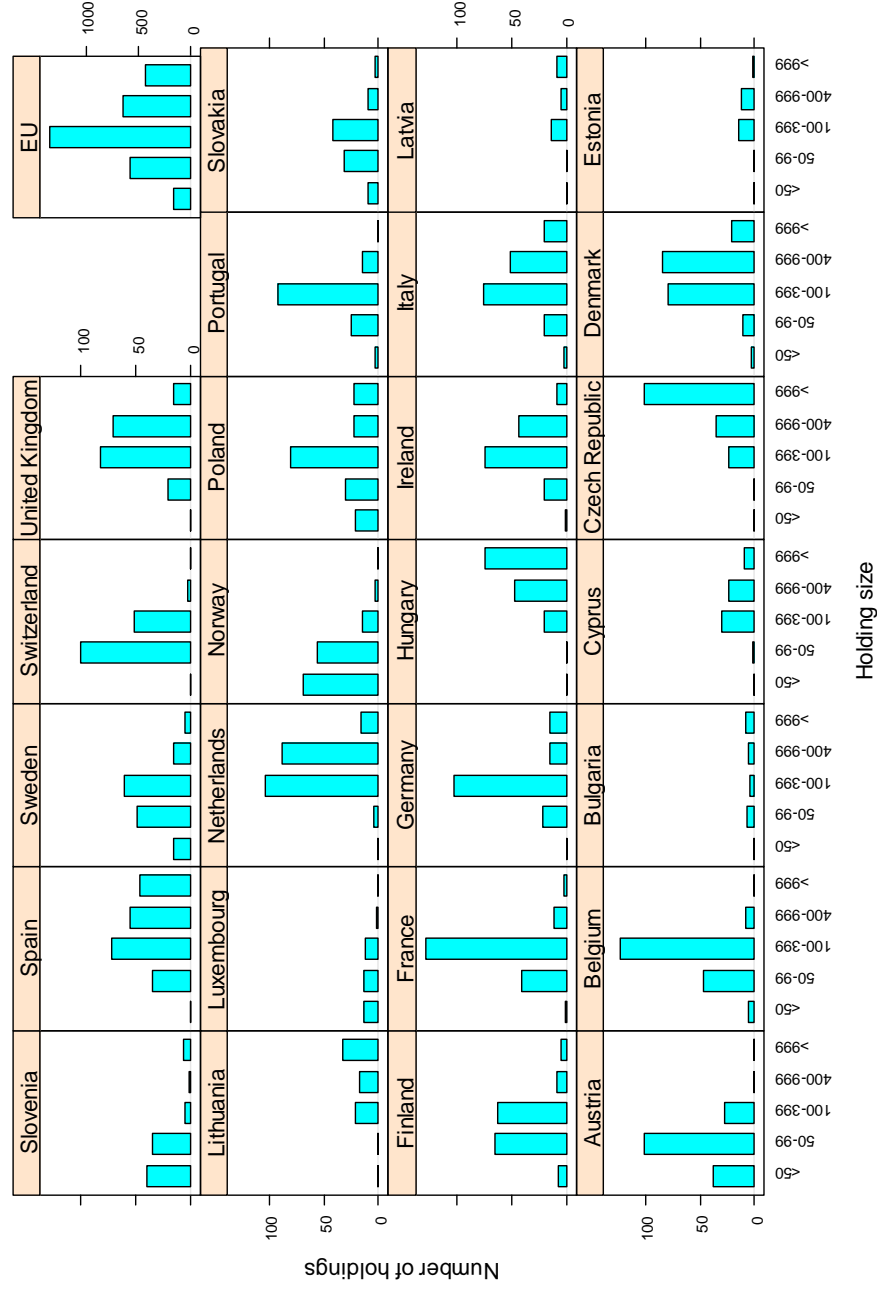


Figure 21: Distribution of the number of sampled production holdings with breeding pigs by the size of the holding, MRSA EU baseline survey in breeding pigs, 2008^(a)

(a): Greece, Malta and Romania did not conduct the survey and two non-Ms, Norway and Switzerland, participated.

Table 14: Distribution of the number of pooled environmental dust samples by the number of days of delay between the sampling date and the starting date of detection testing for MRSA, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	Number of days delay between sampling date and starting date of detection																								Total				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	N	%	N	%	N	%	N	%	N	%		N	%		
Austria	3	1.24	93	38.43	92	38.02	8	3.31	25	10.33	11	4.55	7	2.89	3	1.24											242		
Belgium											22	11.06	48	24.12	49	24.62	2	1.01	3	1.51			6	3.02	35	17.59	34	17.09	199
Bulgaria	8	11.11	25	34.72	15	20.83	14	19.44	3	4.17			2	2.78	5	6.94											72		
Cyprus	1	1.45							1	1.45	6	8.70	11	15.94	13	18.84	14	20.29	1	1.45	3	4.35	3	4.35	6	8.70	10	14.49	69
Czech Republic	42	15.73	127	47.57	62	23.22	21	7.87	8	3.00	4	1.50					1	0.37	2	0.75							267		
Denmark	1	0.34	56	19.11	13	4.44	3	1.02	27	9.22	46	15.70	57	19.45	72	24.57	11	3.75			1	0.34	2	0.68	1	0.34	3	1.02	293
Estonia											1	2.94	4	11.76	12	35.29	5	14.71					8	23.53	4	11.76		34	
Finland									6	3.03	28	14.14	62	31.31	64	32.32	31	15.66	4	2.02							3	1.52	198
France	44	21.89	87	43.28	28	13.93	5	2.49	4	1.99	2	1.00	3	1.49	12	5.97	4	1.99	4	1.99	6	1.75	2	0.58	1	0.29	1	0.29	342
Germany	34	18.78	114	62.98	26	14.36	6	3.31	1	0.55																			
Hungary	5	2.65	127	67.20	37	19.58	4	2.12	8	4.23	4	2.12	2	1.06	2	1.06													
Ireland	1	0.47	3	1.40	26	12.15	14	6.54	20	9.35	10	4.67	17	7.94	22	10.28	23	10.75	9	4.21	9	4.21	14	6.54	9	4.21	37	17.29	214
Italy	4	12.12	14	42.42	10	30.30	1	3.03	4	12.12																		33	
Latvia																													
Lithuania	16	19.51	60	73.17	6	7.32																						82	
Luxembourg																												44	
Poland	1	0.31			2	0.62	6	1.87	4	1.25	34	10.59	103	32.09	112	34.89	12	3.74	7	2.18	11	3.43	3	0.93	5	1.56	21	6.54	321
Portugal	2	1.18	49	28.82	45	26.47	18	10.59	26	15.29	2	1.18	8	4.71	4	2.35	5	2.94	5	2.94	2	1.18	2	1.18	2	1.18		170	
Slovakia	41	21.35	65	33.85	28	14.58	7	3.65	14	7.29	4	2.08	14	7.29	6	3.13	9	4.69	1	0.52	1	0.52				2	1.04	192	
Slovenia	1	0.88	20	17.54	33	28.95	4	3.51	27	23.68	17	14.91	7	6.14	4	3.51	1	0.88										114	
Spain																													
Sweden	6	2.97	4	1.98	3	1.49	1	0.50	5	2.48	13	6.44	89	44.06	64	31.68	12	5.94	2	0.56	172	47.91	133	37.05	20	5.57	9	2.51	359
Netherlands	64	19.94	222	69.16	34	10.59	1	0.31																			5	2.48	202
United Kingdom																												321	
European Union	274	5.96	1 097	23.86	540	11.75	135	2.94	204	4.44	272	5.92	523	11.38	622	13.53	268	5.83	64	1.39	210	4.57	177	3.85	84	1.83	127	2.76	4 597
Norway	8	3.19	165	65.74	49	19.52	25	9.96	4	1.59																		251	
Switzerland	4	1.78	176	78.22	8	3.56	34	15.11	1	0.44	1	0.44																225	

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

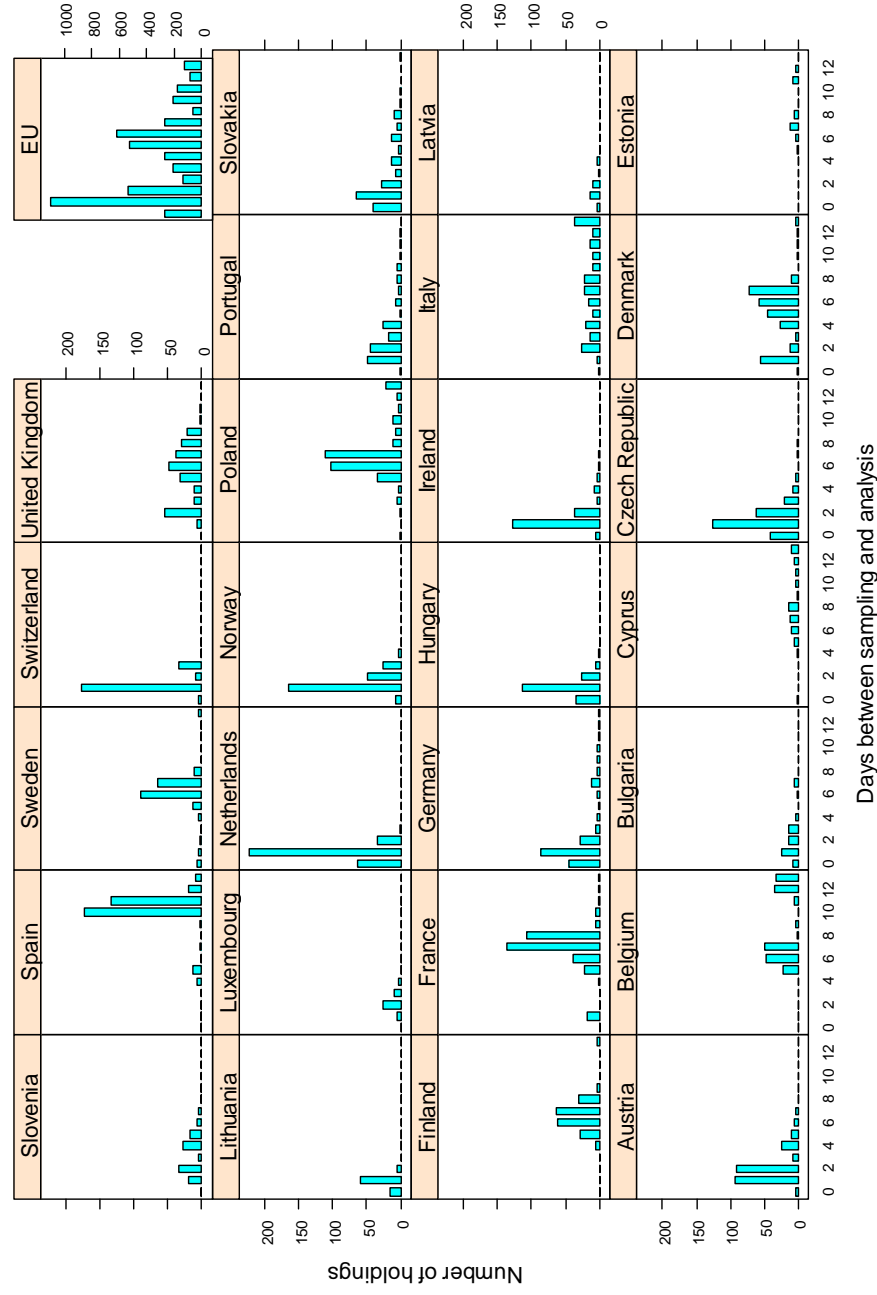


Figure 22: Distribution of the number of pooled environmental dust samples (= number of holdings) by the number of days delay between the sampling date and the starting date of detection testing for MRSA, MRSA EU baseline survey in breeding pigs, 2008^(a)

(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

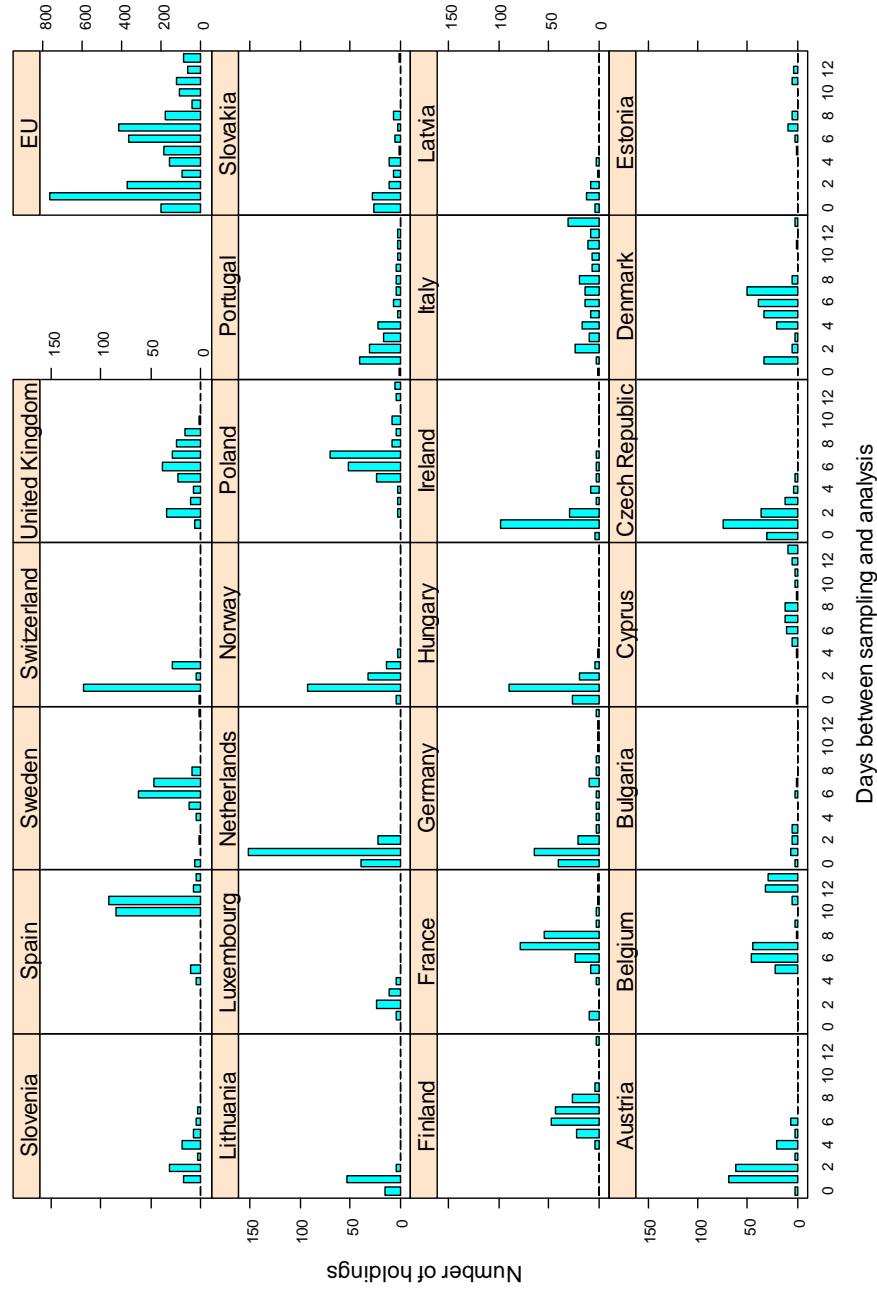


Figure 23: Distribution of the number of pooled environmental dust samples (= number of holdings) by the number of days delay between the sampling date and the starting date of detection testing for MRSA, in breeding holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

(a): Greece, Malta and Romania did not conduct the survey and two non-MTs, Norway and Switzerland, participated.

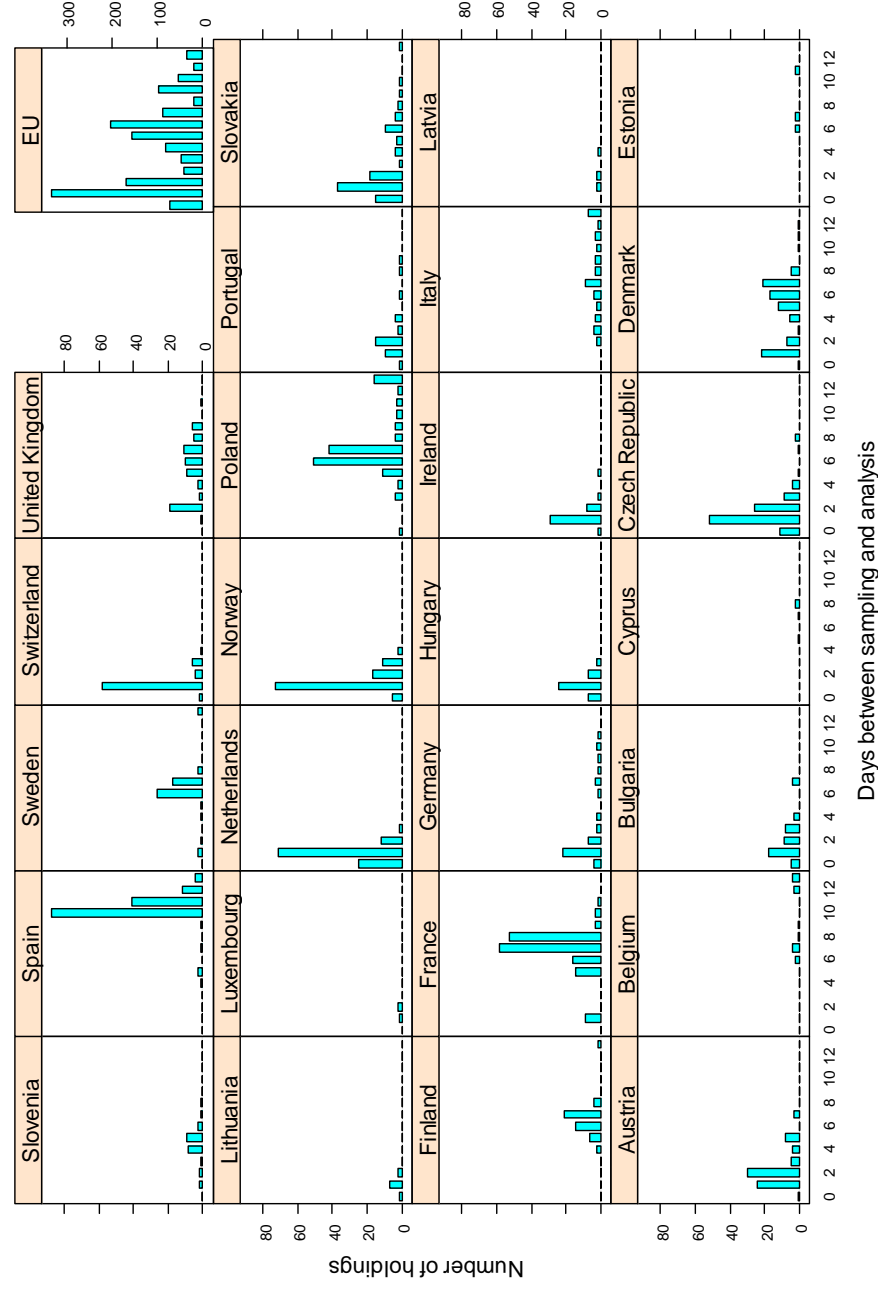


Figure 24: Distribution of the number of pooled environmental dust samples (= number of holdings) by the number of days delay between the sampling date and the starting date of detection testing for MRSA, in production holdings with breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(a)

^(a): Greece, Malta and Romania did not conduct the survey and two non-MTs: Norway and Switzerland, participated.

F. PROPORTION (%) OF MRSA POSITIVE SAMPLED HOLDINGS WITH BREEDING PIGS, IN THE EU AND IN TWO NON-MSS, 2008

Table 15: Number and raw proportions (%) of MRSA positive breeding holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	Total number of holdings	MRSA		ST398		Non-ST398	
		N positive	Raw Pos. %	N positive	Raw Pos. %	N positive	Raw Pos. %
Austria	75	4	5.3	4	5.3	0	0.0
Belgium	15	6	40.0	6	40.0	0	0.0
Bulgaria	47	0	0.0	0	0.0	0	0.0
Cyprus	4	0	0.0	0	0.0	0	0.0
Czech Republic	106	2	1.8	1	0.9	0	0.0
Denmark	95	0	0.0	0	0.0	0	0.0
Estonia	6	0	0.0	0	0.0	0	0.0
Finland	48	0	0.0	0	0.0	0	0.0
France	157	3	1.9	3	1.9	0	0.0
Germany	46	20	43.4	20	43.4	0	0.0
Hungary	40	0	0.0	0	0.0	0	0.0
Ireland	40	0	0.0	0	0.0	0	0.0
Italy	43	15	34.8	6	13.9	9	20.9
Latvia	5	0	0.0	0	0.0	0	0.0
Lithuania	10	0	0.0	0	0.0	0	0.0
Luxembourg	3	0	0.0	0	0.0	0	0.0
Poland	143	3	2.0	3	2.0	0	0.0
Portugal	34	5	14.7	5	14.7	0	0.0
Slovakia	96	1	1.0	1	1.0	0	0.0
Slovenia	27	3	11.1	3	11.1	0	0.0
Spain	150	69	46.0	69	46.0	0	0.0
Sweden	55	0	0.0	0	0.0	0	0.0
Netherlands	109	14	12.8	14	12.8	0	0.0
United Kingdom	67	0	0.0	0	0.0	0	0.0
European Union	1 421	145	10.2	135	9.5	9	0.6
Norway	108	0	0.0	0	0.0	0	0.0
Switzerland	71	0	0.0	0	0.0	0	0.0

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSS, Norway and Switzerland, participated.

Table 16: Number and raw proportions (%) of MRSA positive production holdings with breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(a)

Member State	Total number of holdings	MRSA		ST398		Non-ST398	
		N positive	Raw Pos. %	N positive	Raw Pos. %	N positive	Raw Pos. %
Austria	167	21	12.5	21	12.5	0	0.0
Belgium	184	66	35.8	66	35.8	0	0.0
Bulgaria	25	0	0.0	0	0.0	0	0.0
Cyprus	65	1	1.5	0	0.0	1	1.5
Czech Republic	161	2	1.2	2	1.2	0	0.0
Denmark	198	7	3.5	6	3.0	0	0.0
Estonia	28	0	0.0	0	0.0	0	0.0
Finland	150	1	0.6	1	0.6	0	0.0
France	185	5	2.7	4	2.1	1	0.5
Germany	155	64	41.2	58	37.4	6	3.8
Hungary	141	3	2.1	3	2.1	0	0.0
Ireland	149	0	0.0	0	0.0	0	0.0
Italy	171	58	33.9	37	21.6	21	12.2
Latvia	28	0	0.0	0	0.0	0	0.0
Lithuania	72	0	0.0	0	0.0	0	0.0
Luxembourg	41	15	36.5	15	36.5	0	0.0
Poland	178	6	3.3	6	3.3	0	0.0
Portugal	136	16	11.7	16	11.7	0	0.0
Slovakia	96	0	0.0	0	0.0	0	0.0
Slovenia	87	5	5.7	5	5.7	0	0.0
Spain	209	107	51.1	105	50.2	2	0.9
Sweden	147	0	0.0	0	0.0	0	0.0
Netherlands	212	39	18.3	38	17.9	1	0.4
United Kingdom	191	0	0.0	0	0.0	0	0.0
European Union	3 176	416	13.1	383	12.1	32	1.0
Norway	143	1	0.6	0	0.0	1	0.6
Switzerland	154	0	0.0	0	0.0	0	0.0

(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

G. FREQUENCY DISTRIBUTION OF MRSA *SPA*-TYPES IN HOLDINGS WITH BREEDING PIGS

Table 17: Frequency distribution of *Spa*-types of MRSA in environmental dust samples collected from breeding holdings, MRSA EU baseline survey in breeding pigs, 2008^(a)

		N	%
Austria	t011	4	100.0
	Total isolates	4	100.0
Belgium	t011	5	83.3
	t034	1	16.7
	Total isolates	6	100.0
Czech Republic	t4659	1	50.0
	Non-typeable	1	50.0
	Total isolates	2	100.0
France	t034	1	33.3
	t899	1	33.3
	t2370	1	33.3
	Total isolates	3	100.0
Germany	t011	15	75.0
	t034	3	15.0
	t108	1	5.0
	t2510	1	5.0
	Total isolates	20	100.0
Italy	t127	5	33.3
	t899	4	26.7
	t1730	4	26.7
	t011	1	6.7
	t2922	1	6.7
	Total isolates	15	100.0
Netherlands	t011	7	50.0
	t108	7	50.0
	Total isolates	14	100.0
Poland	t011	3	100.0
	Total isolates	3	100.0
Portugal	t108	3	60.0
	t011	2	40.0
	Total isolates	5	100.0
Slovakia	t034	1	100.0
	Total isolates	1	100.0
Slovenia	t011	2	66.7
	t034	1	33.3
	Total isolates	3	100.0
Spain	t011	49	71.0
	t108	10	14.5
	t1197	4	5.8
	t1255	2	2.9
	t2329	2	2.9
	t1344	1	1.4
	t1456	1	1.4
	Total isolates	69	100.0

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

Table 18: Frequency distribution of *spa*-types of MRSA in environmental dust samples collected from production holdings with breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(a)

		N	%
Austria	t011	20	95.2
	t034	1	4.8
	Total isolates	21	100.0
Belgium	t011	60	90.9
	t034	2	3.0
	t567	2	3.0
	t1451	1	1.5
	t2370	1	1.5
	Total isolates	66	100.0
Cyprus	t127	1	100.0
	Total isolates	1	100.0
Czech Republic	t034	1	50.0
	t2346	1	50.0
	Total isolates	2	100.0
Denmark	t034	7	100.0
	Total isolates	7	100.0
Finland	t034	1	100.0
	Total isolates	1	100.0
France	t011	2	40.0
	t002	1	20.0
	t034	1	20.0
	t899	1	20.0
	Total isolates	5	100.0
Germany	t011	41	64.1
	t034	9	14.1
	t108	4	6.3
	t007	2	3.1
	t1451	2	3.1
	t3992	2	3.1
	t1250	1	1.6
	t1430	1	1.6
	t2510	1	1.6
	t5487	1	1.6
	Total isolates	64	100.0
Hungary	t011	2	66.7
	t1793	1	33.3
	Total isolates	3	100.0

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

Table 18 (contd.): Frequency distribution of *Spa*-types of MRSA in environmental dust samples collected from production holdings with breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(a)

		N	%
Italy	t127	14	24.1
	t899	14	24.1
	t034	10	17.2
	t011	4	6.9
	t1730	4	6.9
	t1939	3	5.2
	t108	2	3.4
	t426	2	3.4
	t2922	2	3.4
	t571	1	1.7
	t2112	1	1.7
	t4838	1	1.7
	Total isolates	58	100.0
Luxembourg	t011	15	100.0
	Total isolates	15	100.0
Netherlands	t011	26	66.7
	t108	10	25.6
	t1403	1	2.6
	t1457	1	2.6
	t3479	1	2.6
	Total isolates	39	100.0
Norway	t008	1	100.0
	Total isolates	1	100.0
Poland	t011	4	66.7
	t034	2	33.3
	Total isolates	6	100.0
Portugal	t108	9	56.3
	t011	5	31.3
	t1255	1	6.3
	t4854	1	6.3
	Total isolates	16	100.0
Slovenia	t011	4	80.0
	t108	1	20.0
	Total isolates	5	100.0
Spain	t011	82	76.6
	t108	13	12.1
	t1197	4	3.7
	t127	2	1.9
	t034	1	0.9
	t567	1	0.9
	t1451	1	0.9
	t2329	1	0.9
	t2330	1	0.9
	t4872	1	0.9
	Total isolates	107	100.0

^(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

H. PHYLOGENETIC ANALYSIS OF THE RELATIONSHIP BETWEEN THE *spa*-TYPES ISOLATED IN BREEDING HOLDINGS AND IN PRODUCTION HOLDINGS WITH BREEDING PIGS

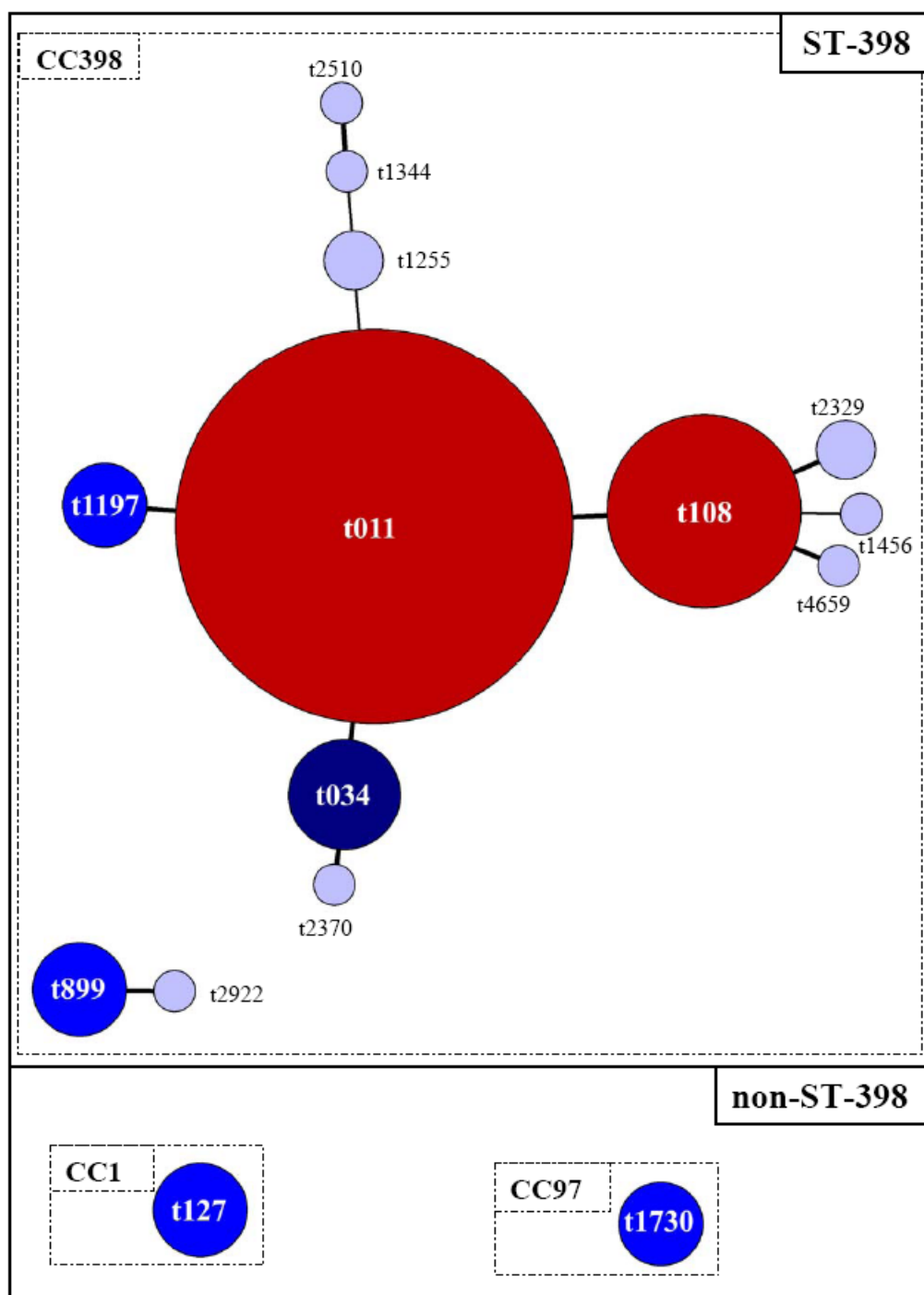


Figure 25: Phylogenetic analysis^(a) of the relationship between the *spa*-types isolated in breeding holdings, MRSA EU baseline survey in breeding pigs, 2008^(b)

(a): For analysis, the minimal Spanning tree algorithm was used with the settings: gap creation cost: 50%; gap extension cost: 50%; duplication creation cost: 25%; duplication extension: 25%; maximum duplication length: 3 re; bin group distance: 50%.

(b): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

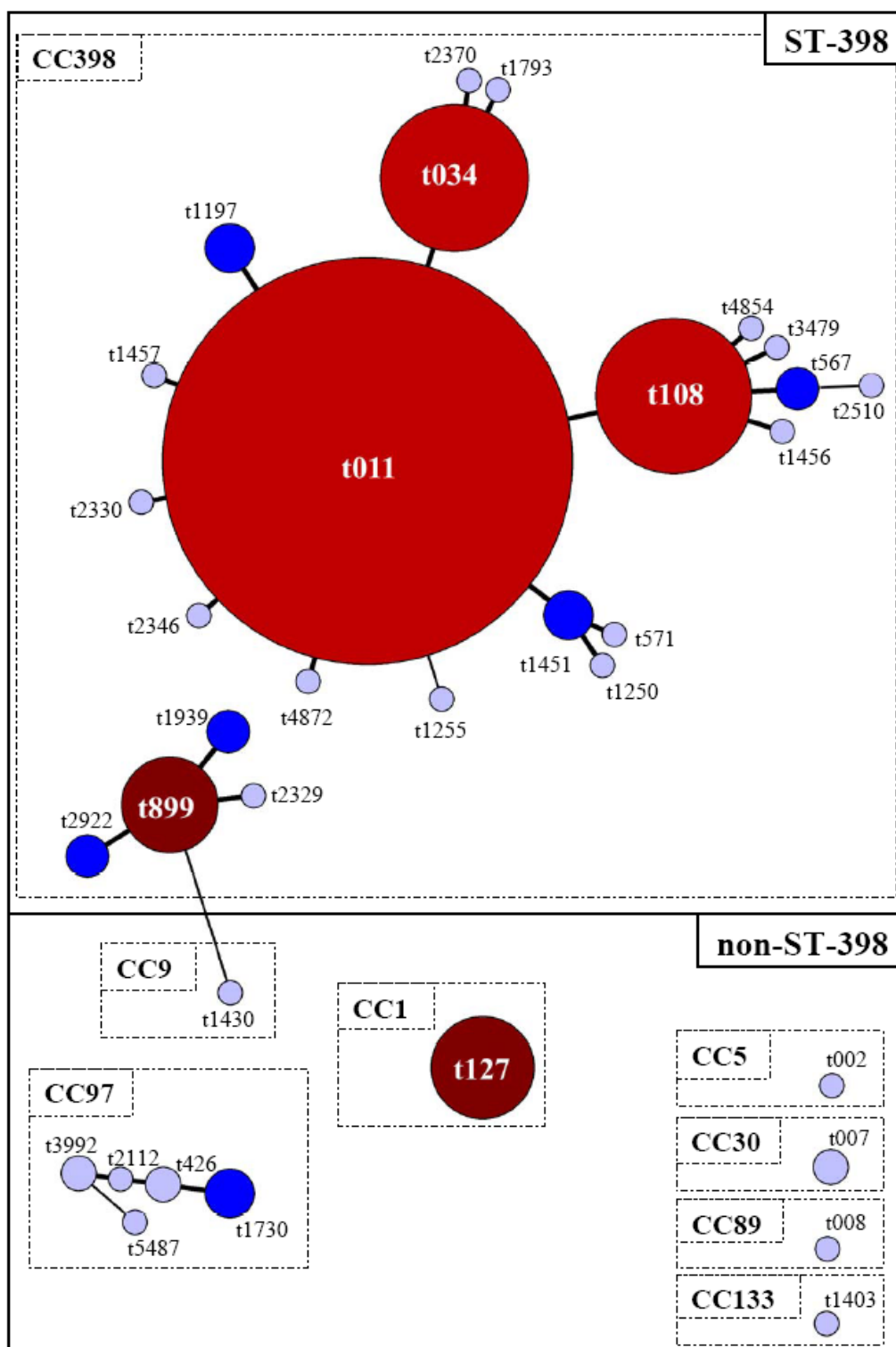


Figure 26: Phylogenetic analysis^(a) of the relationship between the *spa*-types isolated in production holdings with breeding pigs, MRSA EU baseline survey in breeding pigs, 2008^(b)

(a): For analysis, the minimal Spanning tree algorithm was used with the settings: gap creation cost: 50%; gap extension cost: 50%; duplication creation cost: 25%; duplication extension: 25%; maximum duplication length: 3 re; bin group distance: 50%.

(b): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland participated.

I. MRSA NON-ST398 HOLDING PREVALENCE, IN BREEDING HOLDINGS

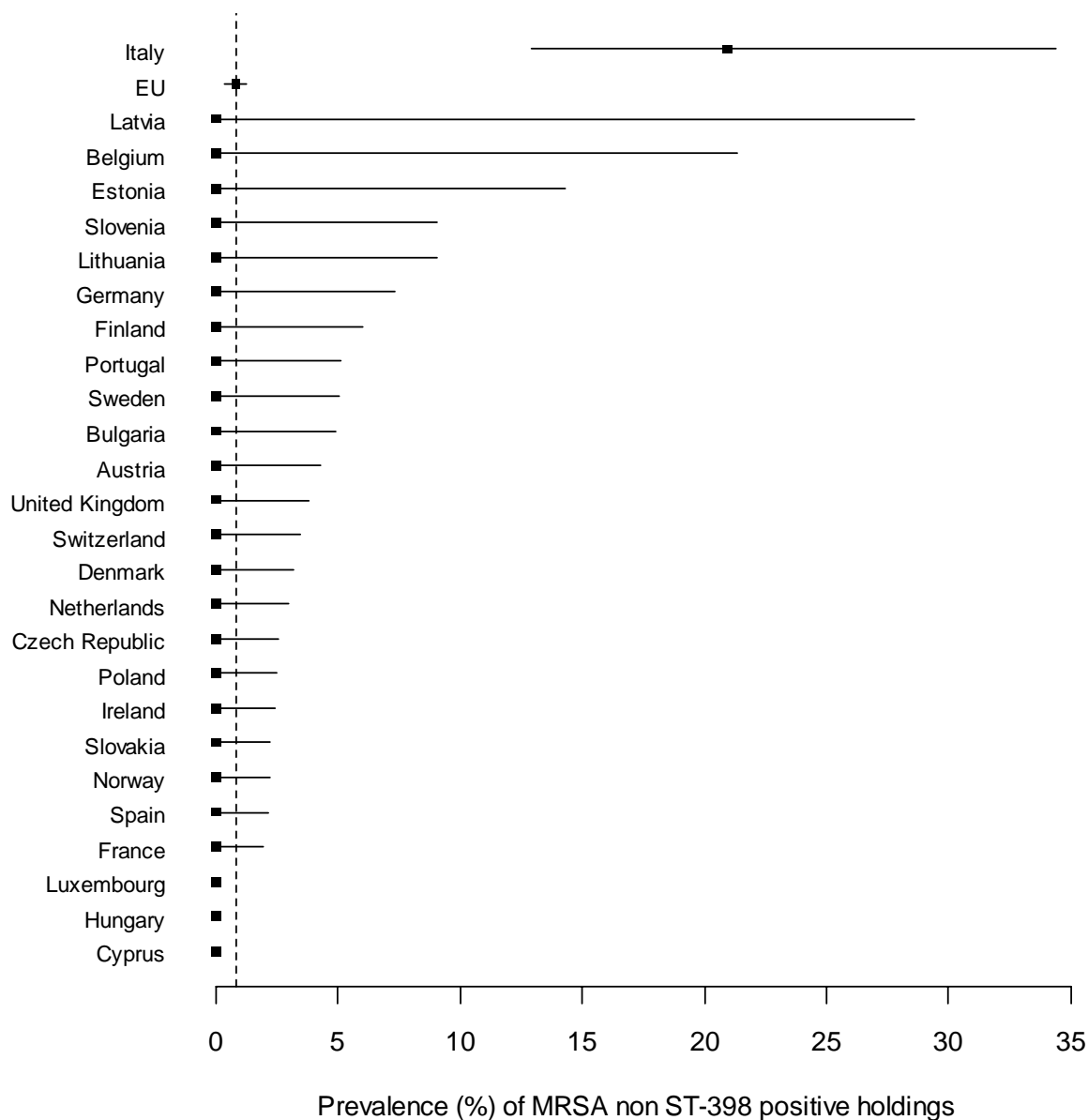


Figure 27: Prevalence^(a) of breeding holdings positive for MRSA non-ST398, with 95% CIs^(b), MRSA EU baseline survey in breeding pigs, 2008^(c)

(a): Horizontal bars represent 95% CIs.

(b): As all existing breeding holdings were included in the survey in Cyprus, Hungary, and Luxembourg (census sampling), the 95% CI based on a finite population approach cannot be calculated and therefore no CI is displayed, although the true CI is likely to be larger.

(c): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

GLOSSARY

Allele	Alternative forms of a gene occupying the same locus on a chromosome. Each of the different states found at a polymorphic site.
Prevalence, apparent, observed and true	Observed prevalence, apparent prevalence or measured prevalence mean the prevalence estimated on the basis of a diagnostic test used to detect the infection in the given population. In contrast, true prevalence represents the actual prevalence of the infection in the population in question. True prevalence can be estimated from the apparent/observed prevalence by correcting for misclassification bias due to the imperfect diagnostic test used. The discrepancy between the apparent and the true prevalence is function of the sensitivity and the specificity of the diagnostic test used.
Boar	A male pig more than six months old and destined for use as a sire.
Breeding holding	Breeding holding means a holding having pigs retained for breeding purposes, covering both nucleus holdings and multiplier holdings. Breeding holdings produce and sell pigs mainly for breeding purposes. The nucleus holdings generate genetic improvement of pure-bred pigs to render them better adapted to the requirements of farmers, processors and consumers, and deliver future pure-bred breeding pigs to multiplier holdings. Multiplier holdings produce future hybrid breeding pigs and deliver them to the production farms with a breeding herd.
Clonal complexes (CC)	The <i>S. aureus</i> population including MRSA consists of different clonal lineages, also called clonal complexes. To determine which lineage an isolate belongs to, the sequence type has to be determined by a method called Multi Locus Sequencing Typing. Closely related STs are grouped into the same CC by the web-based computer software called eBURST.
Community-acquired MRSA	MRSA infection/colonisation acquired outside the hospital and health care settings and without risk factors for HA-MRSA.
Farrow	The act of parturition in the sow.
Farrow-to-finish holding	A pig holding consisting of a herd of sows and their piglets, which are born, reared, weaned, grown and fattened in the one holding.
Farrow-to-grower holding	A pig holding consisting of a herd of sows and their piglets, which are born, reared, weaned and grown to an approximate age of 23 weeks in the one holding and then moved to the care of specialist fatteners.
Farrow-to-weaner holding	A pig holding consisting of a herd of sows and their piglets, which are born and reared up to weaning in the one holding, and then moved to the care of specialist growers and fatteners.
Gilt	A gilt is a female breeding pig that has not yet had a litter of piglets.

Hospital-acquired MRSA	MRSA infection/colonisation acquired in health care settings and which emerges at least 48 hour after admission.
Multi-Locus Sequence Typing (MLST) ⁵	Multi-Locus Sequence Typing is a molecular typing method that allows the checking for nucleotide differences between isolates of microbes. In the case of <i>Staphylococcus aureus</i> , including MRSA strains, it is used to identify the seven housekeeping genes present in all <i>S. aureus</i> strains. This technique involves the sequencing of defined sections of those seven genes, and their comparison using a publicly available database (www.mlst.net). MLST enables the assignment of sequence types to each MRSA.
Multiplier holding	Multiplier holding or supplier holding means a holding of pure-bred pigs that usually produce crossbred future breeding pigs for production holdings.
Nucleus holding	Nucleus holding or pure-bred holding means a holding of pure-bred pigs that produces pure-bred breeding pigs (pure-bred gilts and boar) for multiplier and production holdings.
Phylogenetic analysis	Analysis of the evolution and relation among various groups of organisms (e.g., species, populations), which is discovered through molecular sequencing data and morphological data matrices.
Production holding	Production holdings cover farrow-to-weaner holdings or farrow-to-grower holdings or farrow-to-finish holdings. Production holdings house breeding pigs and sell mainly pigs for fattening to other specialised holdings or for slaughter.
RIDOM StaphType Database (www.spaSpaserver.ridom.de)	<p>Single locus DNA-sequencing of the repeat region of the <i>Staphylococcus</i> protein A gene (<i>spa</i>) can be used for reliable, accurate and discriminatory typing of MRSA. Repeats are assigned a numerical code and the <i>spa</i>-type is deduced from the order of specific repeats. However, <i>spa</i>-typing was hampered in the past by the lack of a consensus on assignments of new <i>spa</i>-repeats and <i>spa</i>-types.</p> <p>This SpaServer can be used to collate and harmonise data from various geographic regions. This WWW site (spaServer.ridom.de) is freely accessible to internet users and the <i>spa</i>-repeat sequences and the <i>spa</i>-types can be downloaded. Chromatograms of new <i>spa</i>-repeats and/or -types can be submitted online for inclusion into the reference database.</p>
Sensitivity	Ability of a test to correctly detect epidemiological units (e.g. animals, holdings) with the disease or infection of interest.
Sow	A female pig that has had a litter.

⁵ A more comprehensive description of genotypic methods most commonly used for the epidemiological characterisation of MRSA can be found in the EFSA opinion on the assessment of the Public Health significance of Methicillin-resistant *Staphylococcus aureus* (MRSA) section 1.7.7. (EFSA 2009).

<i>Spa</i> -typing ⁶	A molecular typing method used for the sub-typing of <i>S. aureus</i> including MRSA and subsequently for identifying MRSA lineage. This technique involves PCR amplification and sequencing of the variable region of the protein A (<i>spa</i>) gene, which encodes the staphylococcal protein A located on the chromosome of all <i>S. aureus</i> . The variable region of the <i>spa</i> gene consists of specific repeated DNA sequences (called “repeats”). The <i>spa</i> -typing method assigns alpha-numerical codes to different repeats, and based on their order and composition, <i>spa</i> repeat sequences are automatically assigned a <i>spa</i> -type by submission to the RIDOM StaphType Database (www.spaSpaserver.ridom.de). The most likely sequence type of new <i>spa</i> -types can often be inferred by comparing to well-defined <i>spa</i> -types with close <i>spa</i> repeat homology for which the ST has already been determined by MLST typing. MRSA with the same <i>spa</i> -type will, in most cases, belong to the same sequence type/clonal complex.
Specificity	Ability of a test to correctly detect individuals free of the disease or infection of interest.
Test misclassification bias	Quality of a test with erroneous classification reflecting its tendency to produce a consistent (directional) deviation from the true state.
Weaner	A young piglet being removed from the sow to switch from sow’s milk to a dry feed.

⁶ A more comprehensive description of the genotypic methods most commonly used for the epidemiological characterisation of MRSA can be found in the EFSA opinion on the assessment of the Public Health significance of Methicillin-resistant *Staphylococcus aureus* (MRSA) section 1.7.7. (EFSA 2009).

ABBREVIATIONS

CC	Clonal Complexes
CI	Confidence Interval
CRL-AR	Community Reference Laboratory for Antimicrobial Resistance
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
MLST	Multi-Locus Sequence Typing
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
MS	Member State
MSSA	Methicillin-Sensitive <i>Staphylococcus aureus</i>
NRL	National Reference Laboratory
PCR	Polymerase Chain Reaction
QA	Quality Assurance
<i>spa</i> -typing	<i>Staphylococcus</i> protein A typing
ST	Sequence type
ST398	MRSA lineage multi-locus sequence type 398
TSB	Tryptone Soy Broth