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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, 2008[1]

Part B: factors associated with MRSA contamination of holdings

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ABSTRACT

A European Union-wide baseline survey on methicillin-resistant *Staphylococcus aureus* (MRSA) was conducted in 2008 in holdings with breeding pigs. A total of 1,600 holdings housing and selling mainly breeding pigs (breeding holdings), and 3,473 holdings housing breeding pigs and selling mainly pigs for fattening or slaughter (production holdings) from 24 Member States and two other European countries were included in the survey. Pooled dust samples collected from holdings were tested for MRSA. The likelihood of a limited set of holding-level factors to be potentially associated with MRSA-positive holdings were analysed by multiple regression analysis, separately for breeding and for production holdings. These analyses showed that the risk of holdings of being contaminated with MRSA increased as the number of breeding pigs in the holding increased, in both breeding and production holdings. Analyses at country-level demonstrated a strong positive association between the prevalence of MRSA-positive breeding holdings and MRSA-positive production holdings, suggesting a vertical dissemination of MRSA between the holdings. A complementary country-level analysis using TRAde Control and Expert System data on intra-Community trade further disclosed strong positive associations between the prevalence of MRSA-positive holdings with breeding pigs and the volume of imported breeding pigs, and a proxy for numbers of imported breeding pigs at risk of being colonised with MRSA. Further investigation of the diversity of MRSA *Staphylococcus* protein A types (*spa*-types) showed that the distribution of *spa*-types differed significantly between countries. *Spa*-types belonging to lineages MRSA ST1, ST5, and ST8 tested negative for Panton-Valentine leukocidin (PVL) toxin genes, suggesting that these strains were not related to the PVL-positive MRSA strains known in human medicine. Further investigations of risk factors for MRSA holding contamination at country level, as well as investigations on prevention and intervention measures to contain MRSA contamination in pig holdings are recommended.

KEY WORDS

MRSA, *spa*-typing, pigs, breeding pigs, baseline survey, risk factors, EU.

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SUMMARY

A European Union-wide survey was carried out in parallel with a baseline survey on *Salmonella* in holdings with breeding pigs to assess the prevalence and diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) in pig primary production and to provide information on potential factors associated with its prevalence. The survey distinguished between breeding holdings (holdings housing breeding pigs and delivering replacement breeding pigs to breeding holdings and production holdings) and production holdings with breeding pigs (holdings housing breeding pigs and producing mainly pigs for fattening or slaughter). The samples for MRSA detection were taken concomitantly in the same holdings that were selected for the *Salmonella* spp. investigation, between January and December 2008. Environmental dust samples were collected from five different pens per holding. These samples were pooled per holding and analysed to determine whether the holding was contaminated with MRSA or not. All isolates were sub-typed by *Staphylococcus* protein A typing (*spa*-typing). A total of 5,073 holdings with breeding pigs with validated results from the European Union (24 Member States), Norway and Switzerland were included in the survey analyses, corresponding to information on 1,600 breeding holdings and 3,473 production holdings. The analysis of the prevalence of MRSA-positive holdings was carried out earlier and was published by the European Food Safety Authority on 24 November 2009 in the Part A report. The present Part B report contains a risk factor analysis for MRSA contamination of breeding and of production holdings, a correlation analysis between MRSA prevalence in breeding and in production holdings, as well as an investigation of the association between intra-Community imports of breeding pigs and MRSA prevalence of a country. In addition, further analyses of the distribution of the *spa*-types of MRSA isolates across Europe, as well as the results of the tests for Panton-Valentine leukocidin (PVL) genes in a subset of isolates originating from the baseline survey, are also included.

Additional data on a limited set of holding-level factors collected by the participating countries as part of the *Salmonella* survey, were investigated with respect to their association with the likelihood of a holding to be MRSA-positive. This included the date of sampling during the year, the types of breeding holdings (nucleus or multiplier) and production holdings (farrow-to-finish, farrow-to-weaner or farrow-to-grower), the size of the holding, the gilt and boar replacement policies of the holding, and the days of delay between sampling and testing in the laboratory. The likelihood of these factors to be potentially associated with MRSA-positive holdings were analysed by multiple regression analysis, separately for breeding and production holdings. The results showed that the risk of holdings of being contaminated with MRSA increased as the number of breeding pigs in the holding increased, both among breeding and production holdings. This effect might reflect a greater risk of introduction and/or of within-holding diffusion of MRSA in larger holdings, for example through a more intensive introduction of replacement breeding stock, but also the impact of other unmeasured underlying risk factors that are associated with structural characteristics and/or managerial practices typical for larger holdings. In addition, the risks of breeding holdings and production holdings being contaminated with MRSA varied significantly between countries even when accounting for the effect of the holding size.

As breeding pigs are intensively traded within and between Member States and may, once colonised, disseminate MRSA to destination holdings, the association between the prevalence of MRSA-positive breeding holdings and the prevalence of MRSA-positive production holdings in a country was investigated and a strong, positive association was observed. Moreover, country-level analyses using TRACES (TRAde Control and Expert System of the Community) data on intra-Community trade of breeding pigs further disclosed a strong, positive association between the prevalence of MRSA-positive holdings and the volume of imported breeding pigs in the country. This association was particularly strong with a proxy for the number of imported breeding pigs that are at risk of being colonised with MRSA. This proxy was obtained by summing the country-of-origin-specific combinations of the number of imported breeding pigs and MRSA holding prevalence. These positive associations are suggesting of a vertical (top-down) dissemination of MRSA in the pig production pyramid within the country and also that the risk of MRSA contamination of holdings with breeding

pigs in a country increases as the volume of imports of breeding pigs from countries with MRSA increases.

An important diversity in MRSA *spa*-types was observed in the European Union, and the *spa*-type distributions of MRSA isolates were found to vary significantly between participating countries. MRSA isolates with *spa*-types belonging to lineages ST1, ST5 and ST8, which are *spa*-types known in human medicine, were tested for PVL toxin genes. None of the tested isolates tested positive for PVL toxin genes, suggesting that those strains were not related to PVL-positive, Community-Associated MRSA strains prevalent in some parts of the world in humans.

Since only a limited set of factors potentially associated with MRSA-positive holdings were analysed in the framework of the survey, it would be useful to perform further national studies to identify more closely the factors that put holdings with breeding pigs at risk of MRSA contamination in specific countries. Also investigations on the impact of addressing MRSA through on-farm biosecurity and managerial practices are needed as a basis for the development of potential, future, measures of prevention and intervention. In particular, it would be important to investigate measures to prevent the introduction of MRSA into MRSA-negative holdings, such as the checking of the MRSA status of replacement breeding pigs. Furthermore, it is advisable to characterise MRSA isolates derived from pigs by using molecular typing methods in order to follow the diversity of the MRSA population.

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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, pig farmers and other human populations. MRSA has been found in both breeding and finishing pig herds in the Netherlands.

Upon a request from the European Commission (EC), 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[4] 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 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 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 European Union (EU) level; and
- to evaluate the risk factors for MRSA in herds of breeding pigs based on the information collected.

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[4] Commission Decision 2008/55/EC of 20 December 2007 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.

ANALYSIS

1. Introduction

This report describes the results of a survey carried out in the EU to estimate the prevalence of MRSA in holdings with breeding pigs. The objective of the survey has been to obtain comparable preliminary data on the occurrence of MRSA for all MSs through a harmonised sampling scheme. The analysis focused particularly on the occurrence of the MRSA lineage ST398, as MRSA ST398 has been recognised as an occupational hazard for people in contact with pigs and can occasionally be introduced into hospitals. This survey was performed in parallel with the baseline survey on *Salmonella* spp. in holdings with breeding pigs during the same visits to selected holdings. In light of 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 the EU, it was acknowledged to be most cost-effective to conduct a preliminary survey evaluating the prevalence of MRSA in holdings with breeding pigs in the EU concomitantly with the survey provided for in Decision 2007/636/EC[5] on the prevalence of *Salmonella* spp. in herds of breeding pigs, which was already planned and close to its launch.

A scientific report of EFSA on the "Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008, part A: MRSA prevalence estimates" (EFSA, 2009b) was published on 24 November 2009. This report included the analyses of the prevalence of MRSA, MRSA ST398 and MRSA non-ST398 in breeding holdings and in production holdings, the most frequent MRSA *spa*-types reported and the phylogenetic analysis of the relationship between the *spa*-types isolated. The survey results indicated that in seven Member States no MRSA was detected in any of the surveyed holdings. The EU prevalence of MRSA-positive breeding holdings was 14.0% and it varied from 0% to 46.0% among MS. The EU prevalence of MRSA-positive production holdings was 26.9%, while in MSs prevalence varied from 0% to 51.2%.

Further data on holding and testing characteristics were collected and reported in the framework of the *Salmonella* baseline survey and thus, were also available to investigate factors potentially associated with MRSA contamination of holdings with breeding pigs. However, as potential explanatory variables were mainly collected at pen level in the *Salmonella* survey, the number of available holding-level variables useful for the analyses of the MRSA survey, was limited. Therefore, the analysis of the effects of potential risk factors for MRSA contamination has to be considered as preliminary.

When assessing MRSA prevalence, the Part A report distinguished between the two types of holdings housing breeding pigs, namely breeding holdings and production holdings with breeding pigs, and provided separate prevalence estimates in these two groups of holdings[6]. In a similar way, as breeding holdings provide breeding pigs to production holdings and may thus vertically disseminate MRSA, separate investigations of the factors associated with MRSA contamination of breeding holdings and of production holdings were therefore considered meaningful in the framework of the risk factor analysis.

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[5] Commission Decision 2007/636/EC 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.

[6] Hereafter in this report, those two types of holding are respectively referred to as 'breeding holdings' and 'production holdings' for brevity. The breeding and production holding types included in the survey are defined in detail in the glossary, and their situations within the pyramidal structure of the pig primary production sector are displayed in Appendix A.

Additionally to the objectives foreseen in Decision 2008/55/EC[7] 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, a further objective to test MRSA belonging to certain sequence types (STs) for PVL toxin genes was included in the analysis. This addition was agreed with the EC and with MSs and other participating countries and it was made because of the public health and scientific interest of these sequence types. PVL is an *S. aureus*-specific exotoxin which can be associated with *S. aureus* strains causing skin and soft tissue infections, as well as severe necrotizing pneumonia (Labandeira-Rey et al. 2007). The MRSA baseline survey revealed (EFSA, 2009b) the presence of some MRSA isolates belonging to STs 1, 5, and 8 in dust samples from pig herds. These STs have been previously detected in humans in the EU, and in some other parts of the world, especially in the North American continent, where some MRSA isolates belonging to those STs are PVL-positive, and are recognised as major causes of CA-MRSA virulence in humans. As PVL-positive MRSA isolates can cause severe invasive infections in man, PVL status is therefore an important consideration in relation to pathogenicity. The ST1, ST5, and ST8 isolates detected in the framework of the MRSA baseline survey were therefore subjected to PVL testing, since the presence or absence of PVL toxins, together with the *spa*-type data, provided useful information on assessing further potential public health significance and could possibly provide some information on lineage/origin.

In analytical studies, the unit of observation is typically the individual of interest, e.g. either animal, herd or holdings. However, all risk factors may not necessarily be at individual level and some of them may be structural risk factors to be looked for at group level such as country level or primary production sector level. Ecological studies are epidemiological studies, in which specific individuals are not studied, but rather groups are compared, e.g. examining differences in the occurrence of MRSA contamination between pig sectors of MSs. As breeding pigs are intensively traded between MSs, and may, once colonised, disseminate MRSA to destination holdings, an ecological descriptive analysis looking at the potential association between the volume of intra-Community trade in breeding pigs and the prevalence of MRSA-positive holdings in MSs was performed in parallel with the risk factor analysis. Inclusion of these additional analyses was agreed with the EC and EFSA's Zoonoses Task Force. Information on the intra-Community trade of breeding pigs was extracted from the TRACES database of the EC (DG Health and Consumers).

The present Part B report contains analyses of the correlation between MRSA prevalence in breeding and in production holdings, and analyses of the effects of potential risk factors for MRSA contamination of breeding and of production holdings. Further analyses of the distribution of the *spa*-types of MRSA isolates across the EU, as well as the results of the tests for PVL genes in a subset of non-ST398 isolates originating from the baseline survey, are also included. Moreover, the association between intra-Community trade and imports of breeding pigs and MRSA prevalence was also investigated. Objectives, sampling frame, diagnostic testing methods, as well as data collection, evaluation, reporting and timelines of the MRSA baseline survey are specified in Commission Decision 2008/55/EC.

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[7] 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 Member States. OJ L 14, 17.1.2008, p.10.

2. Objectives

The primary aim of the survey was to estimate the prevalence of MRSA-positive breeding holdings and of MRSA-positive production holdings, separately, at Community level, as well as for each MS. The previously published Part A report described the results of the analyses of the prevalence of MRSA in breeding holdings and production holdings with breeding pigs. It also reported the most frequent *spa*-types, as well as the phylogenetic analysis of the relationship between the *spa*-types isolated.

The specific objectives related to this Part B report were:

- to investigate the correlation between the prevalence of MRSA-positive breeding and production holdings in each country,
- to investigate the effect of factors, which may be associated with MRSA contamination of breeding holdings and of production holdings, at EU level, and
- to investigate the MRSA *spa*-type distribution in breeding holdings and in production holdings across the EU.

This Part B report also includes complementary descriptive analyses, based on additional information and data. The objectives of these investigations were:

- to investigate the presence of PVL toxin/genes in certain relevant MRSA isolates, and
- to investigate the association between the prevalence of MRSA-positive holdings with breeding pigs and the volume of intra-Community trade of breeding pigs, at country level.

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. Due to scarce data reported, the analyses of the antimicrobial susceptibility of MRSA isolates from the survey will not be addressed in this 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) can be found in Annex I of Commission Decision 2008/55/EC. In addition, an outline of the survey design, the methods of isolation, identification and typing of MRSA, as well as the way in which multi-locus sequence types and clonal complexes were assigned, may also be found in the Part A report (EFSA, 2009b).

3.1. Data description

A detailed description of the validation and cleaning of the dataset carried out was provided in the Part A report (EFSA, 2009b). The final dataset contained data from 5,073 holdings with breeding pigs in 24 MSs, and in two non-MSs (Norway and Switzerland), including 1,600 breeding holdings and 3,473 production holdings with breeding pigs, which formed the basis for all subsequent analyses. All the data, including those from holdings with less than 50 breeding pigs, were included in the report part B analyses. An overview of the validated dataset at holding level is given in Table 1.

In the analysis for this Part B report, Norway and Switzerland are included in the EU level analysis dataset.

Table 1: Overview of the validated data set at holding level with type of breeding and production holdings, MRSA EU baseline survey in breeding pigs, 2008[(a)]

		Breeding holding			**Production holding**			
Country	**No of holdings**	**Nucleus**	**Multiplier or supplier**	**Total**	**Farrow to finish**	**Farrow to grower**	**Farrow to weaner**	**Total**
Austria	242	17	58	75	90	59	18	167
Belgium	199	5	10	15	119	57	8	184
Bulgaria	72	4	43	47	19	1	5	25
Cyprus	69		4	4	65			65
Czech Republic	267	2	104	106	27	124	10	161
Denmark	293	13	82	95	71	52	75	198
Estonia	34	3	3	6	25	1	2	28
Finland	198	15	33	48	51	99		150
France	342	27	130	157	160	6	19	185
Germany	201	11	35	46	59	54	42	155
Hungary	181	12	28	40	131	7	3	141
Ireland	189	6	34	40	132	7	10	149
Italy	214	12	31	43	78	67	26	171
Latvia	33		5	5	28			28
Lithuania	82	7	3	10	39	30	3	72
Luxembourg	44	1	2	3	21	6	14	41
Poland	321	19	124	143	64	102	12	178
Portugal	170	1	33	34	88	29	19	136
Slovakia	192	33	63	96	89		7	96
Slovenia	114	14	13	27	61	26		87
Spain	359	37	113	150	131	78		209
Sweden	202	17	38	55	46	101		147
Netherlands	321	42	67	109	15	193	4	212
United Kingdom	258	32	35	67	128	21	42	191
EU Total	**4,597**	**330**	**1,091**	**1,421**	**1,737**	**1,120**	**319**	**3,176**
Norway	251	40	68	108	104	39		143
Switzerland	225	30	41	71	27	96	31	154

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

3.2. Correlation between the prevalence of MRSA-positive breeding and production holdings

Correlation between the prevalence estimates of MRSA, MRSA ST-398 and MRSA non-ST398 positive breeding and production holdings in each participating country was firstly graphically explored via scatter diagrams and then analysed in a more formal way using the Spearman's rank correlation coefficient.

Secondly, the correlation was also statistically modelled with the aim of accounting for random noise. More detailed information on the statistical model can be found in Appendix E. This second approach was justified by the fact that the assessment of the correlation was based on prevalence estimates of positive breeding and production holdings, meaning that only a sample of existing holdings were investigated in each participating country. Imprecision in the punctual estimates of prevalence (random noise) caused by the sampling process could diminish the strength of the correlation coefficient. The observed correlation as measured by Spearman's rank correlation coefficient therefore underestimated the real value of the correlation and an additional statistical model needed to be fitted.

3.3. Analysis of factors associated with MRSA holding contamination

As previously explained in the Part A report, the prevalence of MRSA-positive holdings 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).

3.3.1. Definition of outcome variables

Data on MRSA contamination in breeding and in production holdings were analysed separately, and the following outcomes were considered:

- holdings contamination with any *spa*-type of MRSA (hereafter MRSA),
- holdings contamination with MRSA ST398, and
- holdings contamination with MRSA non-ST398.

The effect of potential factors associated with MRSA contamination was analysed at holding level. A holding was considered contaminated with MRSA if the microbiological culture of the pooled environmental dust sample detected MRSA, otherwise it was considered negative.

In the Part A report, the prevalence of any MRSA *spa*-types was reported as MRSA and in addition, the prevalence of MRSA ST398 and of MRSA non-ST398 were analysed separately. The analyses for this Part B report also examined each of these outcomes separately. However, as no important differences were observed between the results for the MRSA and MRSA ST398 outcomes, only the results of the analyses of factors associated with the detection of MRSA are presented. Due to limited statistical power because of the low number of cases, no multivariable analyses were carried out for effects of potential factors putting holdings at risk with contamination with MRSA non-ST398 isolates.

3.3.2. Factors to be investigated

Information on factors potentially associated with MRSA holding contamination was collected by the competent authorities or under their supervision at the time of sampling. The mandatory fields in the questionnaire included holding-level factors that could be associated with the outcome variable. The following factors potentially associated with MRSA contamination of breeding holdings and production holdings, described in detail in Appendix B, were considered:

1. Factors related to the sensitivity of the sampling and testing process

- Delay between the sampling date and testing date at the laboratory (Testing delay)

2. Factors related to holding contamination

- Type of breeding/production holdings
- Size of the holding (number of breeding pigs in the holding)
- Gilt replacement policy
- Boar replacement policy

3. Month of sampling / Quarter of sampling

Some additional data on optional holding-level variables were collected on a voluntary basis by reporting countries. However, the effects of these optional factors could not be evaluated due to the scarce data reported. An overview of data reported by MSs is presented in Appendix C. Pen-level variables, available in the framework of the *Salmonella* baseline survey, could neither be used in the MRSA risk factor analysis, as the link between MRSA results and the five pens sampled for MRSA was unknown.

3.3.3. Exploratory analysis of potentially associated factors

Potentially associated factors, which were all categorical variables, were analysed through frequency tables and bar graphs. The association between each potential risk factor and the outcome variable was explored visually by bar graphs of prevalence and 95% confidence intervals (CIs), by different levels of categorical variables. Moreover, a bivariable analysis was performed using a logistic regression model with country as random effect.

3.3.4. Analysis of multicollinearity among potentially associated factors

Data were further analysed for evidence of association among potentially associated factors, since they may be correlated with each other or one may completely explain the association of another (collinearity). The Variance Inflation Factor (VIF) was used as a formal method to detect correlation among risk factors (multicollinearity). Essentially, each potential risk factor is used as the outcome in a regression analysis (described in detail in Appendix D). A VIF value that equals 1 indicates that there is no correlation among risk factors, whereas VIF values higher than 1 indicate a correlation. A VIF value exceeding 10 is interpreted as an indication of strong multicollinearity. Also the correlations of estimated coefficients from the multivariable models were inspected for high values.

3.3.5. Identification of factors associated with MRSA contamination

The multiple regression analysis was applied to obtain estimates of the association between each factor, adjusted for the effect of other factors (potential for confounding)[8] and MRSA contamination of

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[8] In bivariable analysis, a potential risk factor might appear to be associated with MRSA contamination solely because of its association with another risk factor for contamination. If, for example, holdings with breeding pigs from MSs with a higher prevalence of MRSA-positive holdings were mostly sampled during the winter months, winter could result as strongly associated with MRSA prevalence when analysing data at EU level. In this case, conclusions on a strong seasonality of the contamination could be drawn, although it was just the effect of unbalanced sampling. In fact, in this example, season may not have any real effect on MRSA contamination. Confounding is, therefore, the over- or under- estimation of the effect of a potential risk factor due to its association with other risk factors. In the example, the effect of season was overestimated due to the confounding MS effect. In order to eliminate confounding, and to obtain valid estimates of the effect of season, an

breeding holdings or production holdings. Multiple regression analyses were carried out at EU level, including all participating MSs and Norway and Switzerland.

Given the use of a binary outcome variable (MRSA-positive or negative holding status) taking only two, mutually exclusive values (which were coded as "1" when the pooled dust sample was positive and "0" otherwise), logistic regression was the model of choice. However, certain data characteristics needed to be taken into account in the analysis.

The sampling design of the survey was stratified by participating countries, meaning that holdings were sampled and enrolled in the survey from each participating country. Therefore, holdings belonging to the same national pig primary production sector were exposed to similar conditions and to certain same risk factors, including those on which no information was available in the current survey but that might have been associated with MRSA contamination. Holdings belonging to the same pig sector are more likely to be characterised to similar rearing processes, including similar origins of breeders as well as comparable managerial and hygiene practices of farming. It was therefore reasonable to believe that holdings originating from the same participating country could not be considered as independent observations in the statistical analysis. Consequently, correlation among outcomes in those holdings belonging to the same primary production sector was taken into account in the regression models.

For the analysis of potential EU-level risk factors for MRSA holding positivity, a model was fitted where the effect of the country was included as random, resulting in a random intercept logistic regression. The assumption underlying this type of statistical model is that each country, and consequently each holding belonging to that country, was characterised by a certain baseline level of contamination risk, regardless of the exposure to risk factors considered in the survey. These random intercept models consider the population of interest as infinite. This statistical approach, the so-called "model-based" inference (EFSA, 2009c), is different from the Part A report where a "design-based" approach was used to estimate prevalence in a well-specified population. For the Part B report, the random intercept models only consider the prevalence and related risk factors for contamination in all holdings in a country at a certain time estimates of the contamination risk in the country and consider them subject to random variation.

The investigation of the association between factors and MRSA positivity in breeding/production holdings (environmental pooled dust samples) at EU level was carried out using a starting model that contained a global intercept, the factors of interest, and a random intercept for country. This model was reduced by removing stepwise the most non-significant risk factors until only covariates with *P*-values smaller than or equal to 0.05 remained in the final model. The model was fitted using the GLIMMIX procedure in the SAS software. More details on the statistical approach are presented in Appendix D.

As in the Part A report, this Part B presents estimates for MS 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.

3.4. Analysis of the potential association between intra-Community trade in breeding pigs and MRSA-positive holding prevalence

The purpose of this analysis was to explore the potential association between the volume of intra-Community trade in breeding pigs between MSs and the prevalence of MRSA-positive holdings in MSs. This analysis was based on import data gathered from a source that was different from the

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adjustment for MS is necessary, which can be achieved by multiple regression analysis. In certain cases, however, two or more potential risk factors may be so strongly associated that separate estimates of their respective effects cannot be obtained. In this case, the terms collinearity or multicollinearity are used.

baseline survey questionnaire data. Therefore, these trade data were not included in the preceding risk factor analysis, but constituted a separate and specific section of this report Part B.

3.4.1. TRACES data

On EFSA's request, the EC (DG SANCO) kindly provided EFSA with data regarding intra-Community[9] trade of live pigs from 2006 to 2008 extracted from TRACES[10]. Data available were aggregated at country level. TRACES covers information on the trade between EU MSs but does not include information on trade between MSs and countries outside the EU, except Norway and Switzerland. The data included the number of pigs and consignments per category of live pigs, such as breeding, slaughter, and fattening pigs. Only trade data relative to breeding pigs were analysed and this category included both breeding pigs intended for nucleus and multiplier holdings as well as breeding pigs intended for production holdings with breeding pigs, without distinction.

3.4.2. Explorative analysis

First, descriptive maps were produced displaying simultaneously the estimates of MS-specific prevalence of MRSA-positive holdings and the MS-specific volume of intra-Community trade in breeding pigs covered by the TRACES database. Specifically, a network of all possible trading routes among MSs for each year was created to be overlapped on MRSA prevalence maps showing the amount of breeding pig imports and exports among MSs.

Secondly, the correlation between the prevalence of MRSA-positive holdings with breeding pigs in the country and the import volume into a country of breeding pigs (trading data for 2006, 2007 and 2008 as well as pooling information together for all three years) was more formally tested using the Spearman's rank correlation coefficient. The following variables quantifying the import volume of breeding pigs per MS were investigated:

- number of breeding pigs imported,
- proportion (%) of pigs imported (proportion of breeding pigs imported out of the total 2008 pig population),
- number of consignments imported (number of import consignments with breeding pigs), and
- number of trading partners (countries of origin) from which breeding pigs were imported.

Thirdly, a logistic random effect model was fitted to assess the significance of the association between the same above-mentioned variables, which were specified for each country as a fixed effect, and the MRSA contamination of holdings with breeding pigs, of breeding holdings, and of production holdings. In the model the country was specified as random effect. The analysis was done separately for the years 2006, 2007 and 2008 as well as for all three years together.

Lastly, the association between MRSA holding contamination and the import volume into a country of breeding pigs at risk of being colonised with MRSA, was assessed. For the purpose, a proxy-number of imported breeding pigs at risk of being colonised was estimated per year and per MS by summing over all countries of origin, the number of imported breeding pigs from a country of origin multiplied

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[9] Trading data with Third Countries, except Norway, were not considered in this preliminary approach.

[10] TRACES was established by Commission Decision 2004/292/EC of 30 March 2004 on the introduction of the TRACES system and amending Decision 92/486/EEC. TRACES is an integrated web-based veterinary system, maintained by the European Commission (Directorate General Health and Consumer Protection), networking veterinary competent authorities, and business users in all MSs, EFTA/EEA countries and a certain number of third countries. TRACES assists in the management of intra and extra community trade of live animals, animal products and germplasm by providing traders with the relevant certificates (completed and transmitted electronically). Through TRACES, information between all relevant national and Community authorities is coordinated, to provide a rapid reaction in case of an animal disease outbreak. Direct access to the system is restricted due to sensitivity of the data.

by the prevalence of MRSA-positive holdings in the country of origin (see formula in Appendix H). For the latter, prevalence of MRSA-positive holdings as estimated by the baseline survey was used as a proxy of MRSA prevalence in breeding pig holdings for the years 2006, 2007 and 2008. This variable was inserted in a similar logistic random effect model to assess the significance of the association, as described in the previous paragraph.

3.5. Analysis of MRSA *spa*-type distribution across the EU

3.5.1. Spatial distribution of MRSA *spa*-types in the EU

The distribution of *spa*-types was investigated for differences between countries. To this end, a Fisher-exact test was considered. However, calculating the significance of the Fisher-exact test by enumeration was not feasible because of the large amount of data, and a small simulation study, enabling calculations, was conducted instead.

The geographical visualisation of the *spa*-type distribution was limited to country level, as the location (coordinates) of the individual holdings participating in the study was not available. Maps showing the distribution of the most frequently identified *spa*-types among pigs from breeding holdings and production holdings were produced.

3.5.2. PVL toxin analysis

In the framework of the EU-wide MRSA baseline survey, in total 24 isolates of MRSA *spa*-types, i.e. t127 (n=22), t002 (n=1) and t008 (n=1) belonging to the sequence types ST1, ST5 and ST8, respectively, were isolated in holdings with breeding pigs in five participating countries: Italy (n=19), Spain (n=2), Cyprus (n=1), France (n=1), and Norway (n=1). Presence of the PVL toxin genes (LukS-PV/LukP-PV) were investigated by Polymerase Chain Reaction (PCR) according to previously published methods (Lina et al, 1999) in the European Union Reference Laboratory for Antimicrobial Resistance (EU-RL-AR). The PVL positive reference strain ATCC 25923 was used as a positive control in the PCR.

4. Results

4.1. Association between prevalence of MRSA-positive breeding holdings and MRSA-positive production holdings

The scatter diagrams of the prevalence of MRSA and of MRSA ST398 positive breeding holdings versus production holdings are displayed in Figures 1 and 2, respectively. A similar scatter diagram for MRSA non-ST398 is presented in Appendix E. The scatter diagrams show that the prevalence of MRSA-positive production holdings increases as the prevalence of MRSA-positive breeding holdings increases, meaning that there is a positive correlation. This observation is notably clearer for countries with a prevalence above 5% for either breeding or production holdings.

Correlations between the prevalence of MRSA, MRSA ST398, and MRSA non-ST398 positive breeding and production holdings in each participating country were studied formally using the Spearman's rank correlation coefficient ρ, a nonparametric rank correlation procedure which can be used when few data pairs (26) are available. The estimated correlations are presented in Table 2. This table also includes *P*-values from testing the null hypothesis of no association between the prevalence estimates in the two types of holdings. Significant correlation was observed for MRSA, for MRSA ST398 and for MRSA non-ST398 ($P<0.05$). These significant results are based on calculations that also take account of the results from MSs that reported no positive outcomes for both breeding and production holdings.

Table 2: Spearman's correlation coefficients and corresponding *P*-values for the correlation test between the prevalence of MRSA, MRSA ST398, and MRSA non-ST398-positive breeding and production holdings. MRSA, EU baseline survey in breeding pigs, 2008 [(a)]

Prevalence	Spearman ρ	*P*-value
MRSA[(b)]	0.76	<0.0001
MRSA ST398	0.78	<0.0001
MRSA non-ST398	0.43	0.03

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

(b) all *spa*-types.

Correlations estimated by the random effect model with separate country specific random effects for breeding and production holdings turned out to be almost 100% (Appendix E, Table 5).

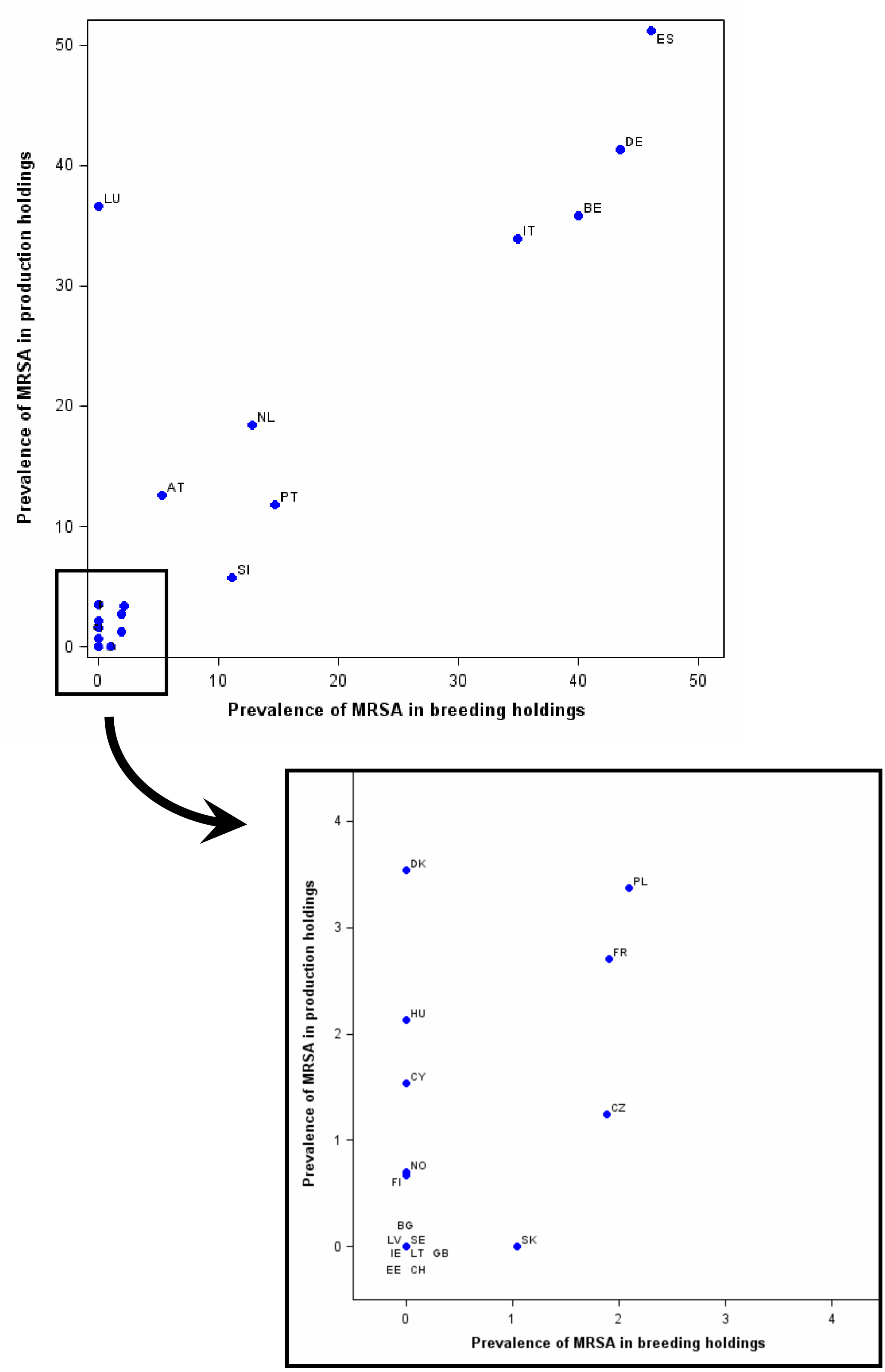


Figure 1: Scatter diagram of the prevalence of MRSA-positive breeding holdings versus MRSA-positive production holdings, MRSA EU baseline survey in breeding pigs, 2008$^{(a)}$

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

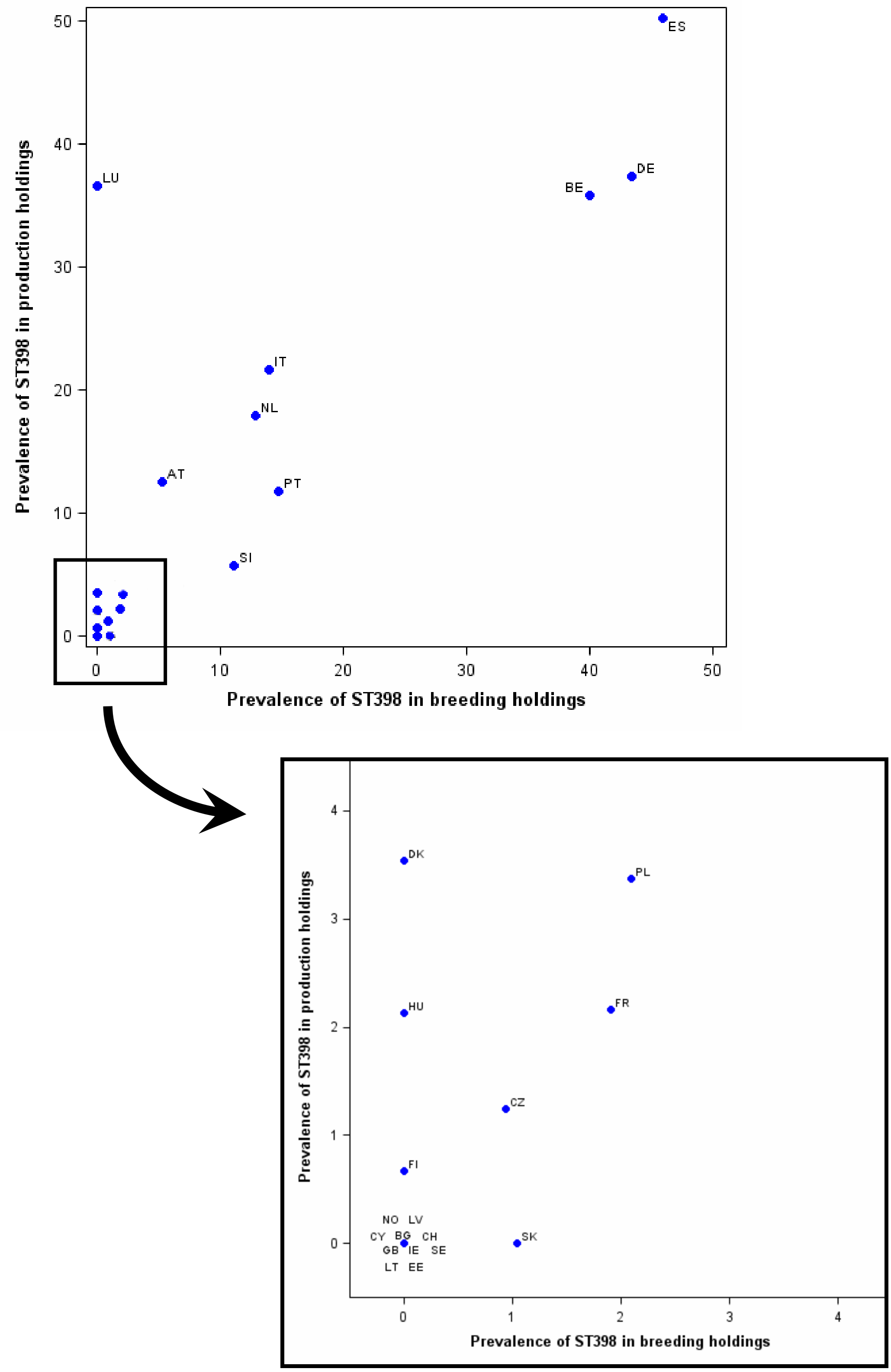


Figure 2: Scatter diagram of the prevalence of MRSA ST-398 positive breeding holdings versus MRSA ST-398 positive production holdings, MRSA EU baseline survey in breeding pigs, 2008 [(a)]

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

4.2. Analysis of factors associated with MRSA holding contamination

In this section the results of the bivariable association between potentially associated factors, and the prevalence of MRSA-positive breeding holdings and production holdings, respectively, are presented. These bivariable analyses are to be considered as exploratory because they do not adjust for the effect of other factors (potential for confounding) and for country effects. Secondly, the results of the bivariable analysis are shown based on a logistic regression model with country as random effect.

4.2.1. Descriptive analysis of factors potentially associated with MRSA contamination

The results of the descriptive bivariable association analyses between potential associated factors and the prevalence of MRSA-positive breeding holdings and production holdings are presented in Appendix F (Tables 6-7, Figures 8-12). The results of the bivariable analysis performed, using logistic regression with country as random effect, are presented in Appendix F, Table 8.

- Testing delay

Overall, for both breeding and production holdings, more holdings tested positive when the delay between sampling and testing was greater than seven days, as compared to samples analysed within seven days after sampling (Appendix F, Figure 8).

- Quarter of sampling

A categorical variable “Quarter of sampling” was created with the following four categories: January-March 2008, April-June 2008, July-September 2008, and October-December 2008. The four categories were coded according to when the holding was sampled: “1” for the period January-March 2008; “2” for April-June 2008; “3” for July-September 2008; and “4” for October-December 2008. The prevalence of both MRSA-positive breeding and production holdings appears to increase towards the end of the survey (Appendix F, Figure 9), even though differences are not statistically significant (Appendix F, Table 8).

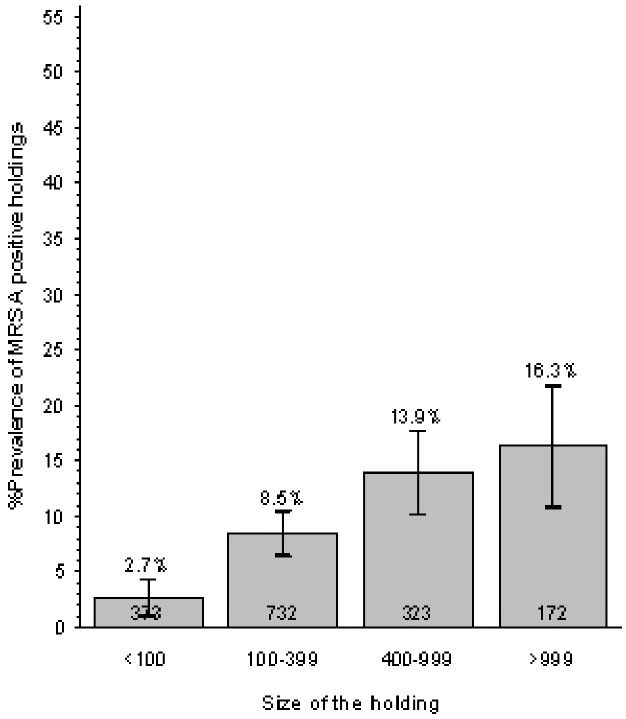
- Type of breeding/production holdings

MRSA prevalence does not seem to differ between the different sub-types of breeding holdings (nucleus and multiplier holdings) and of production holdings (farrow-to-finish, farrow-to-weaner, and farrow-to-grower holdings) (Appendix F, Figure 10). Associations are not significant (Appendix F, Table 8).

- Holding size (number of breeding pigs present in the holding on the date of sampling)

An increase in MRSA holding prevalence was found to be associated with an increased size of the holding for both breeding holdings and production holdings (Figure 3). This effect of holding size, i.e. the number of breeding pigs present in the holding at the date of sampling, is significant in breeding holdings (*P*=0.008) as well as in production holdings (*P*=0.042) (Appendix F, Table 8).

Breeding holding



Production holding

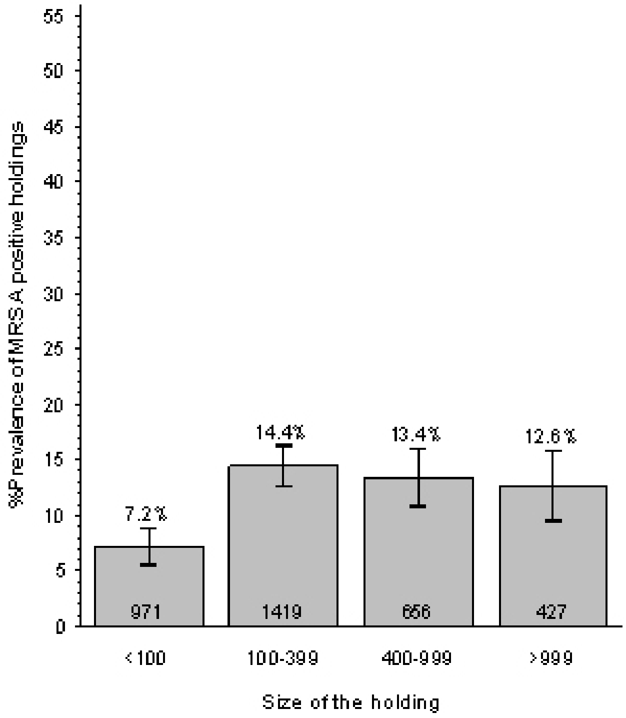


Figure 3: MRSA prevalence in breeding and production holdings with 95% CI by holding size (number of holdings represented inside each bar), MRSA EU baseline survey in breeding pigs, 2008[(a)]

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

- Gilt replacement policy

Graphically, there seems to be no difference between the MRSA prevalence in breeding holdings with '>90% gilts home-bred' and breeding holdings with '>90% gilts purchased'. Whereas, in the case of production holdings, prevalence in production holdings with '>90% gilts purchased' was higher than in production holdings characterised by '>90% gilts home-bred' (Appendix F, Figure 11). However, considering the bivariable model with country as random effect, an effect was also observed in breeding holdings, indicating a higher prevalence in breeding holdings with '>90% gilts purchased' compared to breeding holdings with '>90% gilts home-bred' (Appendix F, Table 8).

- Boar replacement policy

Both in breeding holdings and in production holdings, graphs suggest a slight decreasing trend in prevalence as the percentage of purchased boars increases. However, no major differences in prevalence were disclosed between holdings without boars and those housing boars, whether purchased or home-bred (Appendix F, Figure 12).

4.2.2. Analysis of multicollinearity among potential factors

The results of the VIF analysis, separately for breeding and production holdings, showed that no VIF value was greater than 10. Neither the checking of correlations of estimated coefficients from the multivariable models revealed any high values. This indicated multicollinearity was not important for the starting model.

4.2.3. Multivariable regression analysis at EU level

The same factor associated with MRSA contamination in breeding holdings and in production holdings, which was retained in both the final regression models, was related to holding characteristics. The models, presented in Table 3, included a significant random intercept for the countries.

According to the analyses, the probability of MRSA detection in breeding holdings and in production holdings increased as the size of the holding, as measured by the number of breeding pigs, increased. For example, the odds of detecting MRSA in a breeding holding housing between 400 and 999 breeding pigs is 2.74 times higher than the odds for a holding housing less than 100 breeding pigs.

The country random effect in both models was highly significant ($P<0.0001$) and respectively 65.0% and 63.2% of the total variance in MRSA prevalence could be attributed to a country in the breeding holding and production holding models.

At the penultimate step of the backward stepwise procedure, the variable "gilt replacement policy" was the last variable to be removed from the model for breeding holdings with a *P*-value of 0.065 before fitting the final model. This *P*-value is very close to the significance threshold of 0.05. When adjusted on all the other potential explanatory variables, "gilt replacement policy" appears as significantly associated with MRSA prevalence in the starting model for breeding holdings (full model used as the starting point of the backward stepwise procedure) presented for information in Appendix G. In the starting model, purchasing more than 90% of the gilts increases the likelihood of MRSA detection in breeding holdings by 80% compared to having >90 % home-bred gilts. In the final model this association was not significant.

Table 3: Final random effect logistic models for factors associated with MRSA contamination in breeding holdings and in production holdings, MRSA EU-baseline survey in breeding pigs, 2008$^{(a)}$

Variables	**Final Random effect logistic models**					
	Breeding holdings $^{(b)}$			**Production holdings** $^{(b)}$		
	OR[11]	**95%CI**	***P*-value**	**OR**[11]	**95%CI**	***P*-value**
Holding size (number of breeding pigs)			0.008			0.004
<100	1	-		1	-	
100-399	1.96	0.95, 4.04		1.86	1.14, 3.04	
400-999	2.74	1.29, 5.85		2.22	1.34, 3.66	
>999	2.54	1.33, 4.87		2.58	1.41, 4.70	

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

(b): Estimates and standard errors were assessed using a logistic mixed model with a country random effect on the intercept (*P*-value < 0.0001).

4.3. Association between trade in breeding pigs and MRSA holding contamination

4.3.1. Maps illustrating intra-Community trade of breeding pigs

In this section, results of the analyses of the impact of trade regarding breeding pigs are presented. Maps have been produced to visualise simultaneously the volume of breeding pig imports and exports among MSs in 2006, 2007 and 2008 and MRSA prevalence in the 2008 survey (Figure 4 and Figures 13 and 14 in Appendix H). Furthermore, exports from Norway and Switzerland were also considered. The figures display MRSA prevalence the number of imported pigs as visualised by circles of different sizes, and the trade in breeding pigs as visualised by lines and arrows between exporting and importing countries.

Germany was the country importing the highest number of breeding pigs in the three investigated years (2006, 2007, 2008), followed by, respectively in decreasing order, Spain, Italy, Belgium and Poland in 2006 and 2007. In 2008, Belgium and Poland were the second and third country importing most breeding pigs, followed by Spain and Italy.

The Netherlands was the country exporting the highest number of breeding pigs within the EU, representing the major source of breeding pigs for Germany, Spain and Italy over the three-year period. Denmark accounted for the second highest number of breeding pigs exported within the EU in all the investigated years. Other MSs within the top five countries exporting breeding pigs were Germany, France and Belgium.

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[11] An Odds Ratio (OR) of 1.0 implies that there is no association between a risk factor and MRSA contamination; an OR above 1.0 implies an increased risk of MRSA contamination among holdings exposed to that factor while an OR below 1.0 implies a reduced risk of MRSA contamination among exposed holdings. In any study, it is possible that an OR different to 1.0 may arise by chance and the level of significance (*P*-value) estimates this probability. Consequently, if the 95% CI of the OR does not comprise 1, meaning that both the lower and the upper limits are either greater, or less than 1, it can be concluded that the association with a potential risk factor and MRSA is statistically significant ($P<0.05$).

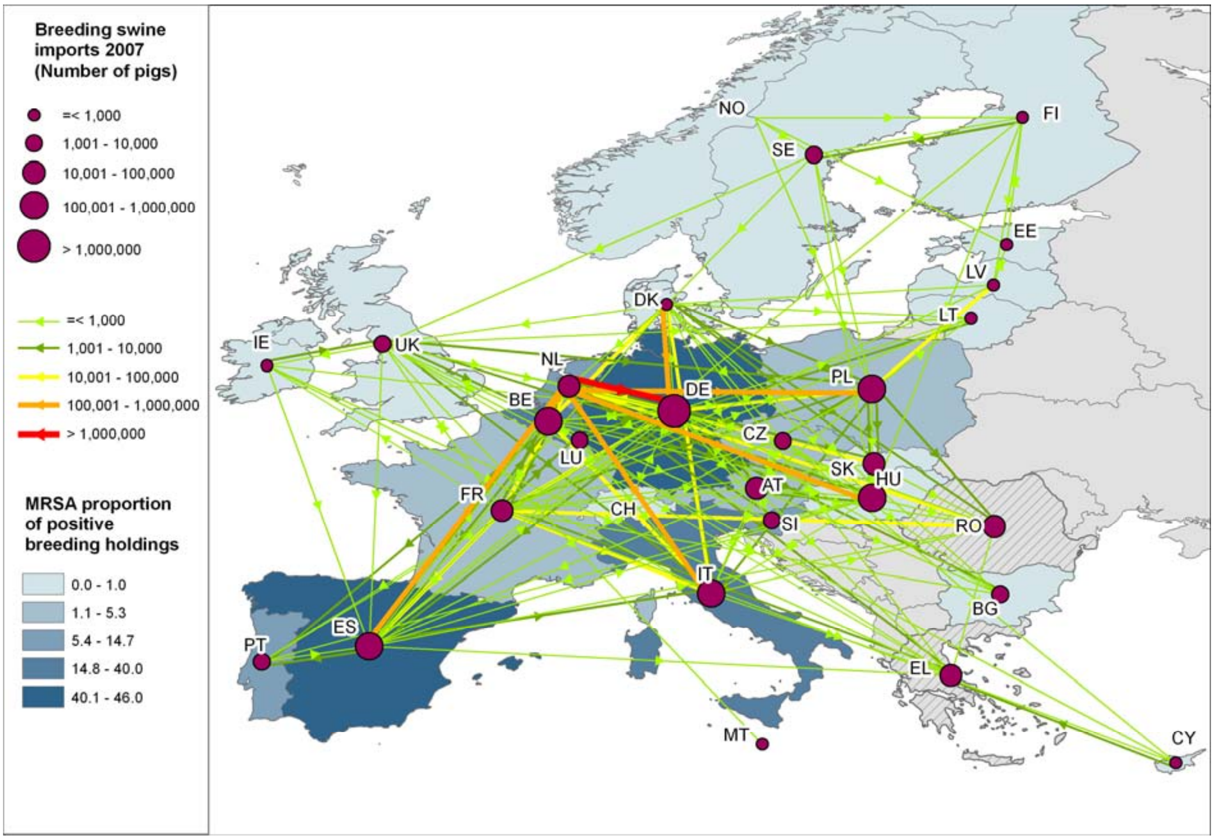

Figure 4: Prevalence of MRSA-positive breeding holdings in 2008 (EFSA, 2009) and intra-Community trade of breeding pigs in 2007.[12]

4.3.2. Correlation between volume of trade in breeding holdings and MRSA holding contamination

- Spearman's correlation coefficient

Correlation analyses using Spearman's correlation coefficient resulted in a significant correlation between the number of breeding pigs imported and the prevalence of MRSA-positive holdings with breeding pigs, in 2008. Significant correlation was also detected between the prevalence of MRSA in 2008 and other variables quantifying trade information, such as the percentage of imported pigs (proportion of breeding pigs imported per MS out of the total pig population), the number of consignments imported (number of shipments of imported breeding pigs per MS), and the number of trading countries from which breeding pigs where imported. Spearman's correlation coefficients were significant when investigating the three years separately (2006, 2007, 2008) as well as when pooling all the three years together. However, values were highest for the year 2007. Results of these correlation analyses (Spearman's coefficients ρ, and *P*-values) are presented in Table 10 (Appendix H).

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[12] Lines connecting Country A and Country B are represented with a specific colour (green to red where red represents larger numbers) and thickness (increasing thickness corresponds to larger numbers) to display the amount of imports among the MSs. Arrows along the route indicate direction of flux. A dot for each country is represented with a specific size based on the total number of breeding pigs imported by each country (larger dots represent larger numbers).

- Correlation by models

The results of the logistic random effect models fitted to assess the significance of the association between imports of breeding pigs and MRSA contamination of holdings are presented in Appendix H and Table 11. The strength of the association is assessed by the percentage of variance explained (R^2 as a percentage) and the significance by a *P*-value lower than 0.05.

Whatever variables used to reflect the import volume of breeding pigs in the country, either by number of trading partners (countries of origin), number of consignments imported, number of pigs imported, or percentage of pigs imported, for the years 2006, 2007, 2008 and for all years combined together, the results of the logistic random effects model confirmed the significant association between the volume of breeding pig imports and the prevalence of MRSA-positive holdings with breeding pigs (all holdings), breeding holdings and production holdings (Appendix H, Table 11), as previously indicated by Spearman's correlation coefficients.

The association between the proxy number of imported breeding pigs at risk of being MRSA colonised and the MRSA frequency in holdings with breeding pigs (all holdings), breeding holdings and production holdings are also positive and highly significant for the years 2006, 2007 and 2008, and for all years combined together (Table 4). The strength and significance of the associations between the prevalence of MRSA contaminated holdings and the proxy-number of imported breeding pigs at risk of being MRSA colonised are generally greater (Table 4) than the strength and significance of associations with the general volumes of breeding pig imports (Table 11).

Table 4: Strength and significance of the association between the prevalence of MRSA contaminated holdings in 2008 and proxy-number of breeding pigs at risk of MRSA colonisation imported in 2006-2008

Holdings	Year	Percent of variance explained	*P*-value
All holdings	2006	31.4%	0.00424
	2007	32.4%	0.00838
	2008	17.6%	0.01504
	2006-2008	31.2%	0.0096
Breeding holdings	2006	42.7%	0.00059
	2007	38.9%	0.00092
	2008	33.7%	0.01389
	2006-2008	36.1%	0.00112
Production holdings	2006	27.8%	0.00464
	2007	31.3%	0.00868
	2008	16.9%	0.01465
	2006-2008	30.0%	0.01004

4.4. Analysis of the MRSA *spa*-types distribution across the EU

4.4.1. Spatial distribution of MRSA *spa*-types in the EU

As a first approach, the Fisher exact test was performed to check whether there is a significant statistical difference in *spa*-type distributions of MRSA among participating countries. The *P*-value was <0.0001, indicating that the proportions of *spa*-types are different between countries.

The distribution of the five most frequently isolated MRSA *spa*-types collected from breeding and production holdings across the EU in 2008 is shown in Figures 5 and 6, respectively. The geographic distribution of MRSA *spa*-types in holdings with breeding pigs (including both breeding and production holdings) across the EU is presented in Appendix I.

MRSA *spa*-type t011 was the most commonly isolated *spa*-type across the EU, being detected in nine out of the 12 positive MSs in breeding holdings and in 12 out of the 16 positive MSs in production holdings. *Spa*-type t108 was isolated in four and in six MSs in breeding and production holdings, respectively. Conversely, *spa*-type t034 was isolated in five and in 10 MSs in breeding and production holdings, respectively. A particular spatial distribution across the EU has been shown for *spa*-types t899 (belonging to CC398) and t127 (belonging to CC1) and the MRSA *spa*-types non-ST-398 other than t127. *Spa*-type t899 was only isolated in Italy (four isolates in breeding holdings and 14 in production holdings) and in France (one isolate in breeding holding and one in production holding). Mapping the distribution of MRSA *spa*-types across the EU allowed a better visualisation of the unique situation in Italy regarding the distribution of non-ST-398 *spa*-types in both breeding and production holdings.

As shown in Figure 5 for breeding holdings, the Italian *spa*-type distribution differed considerably from other EU countries, as *spa*-types belonging to the MRSA non-ST398 group were only isolated in Italian breeding holdings, in particular *spa*-type t127.

The spatial distribution of MRSA *spa*-types in production holdings across the countries was similar to that observed in breeding holdings, although in general a higher number of *spa*-types were isolated in several MSs (Figure 6). At country level, most of the spa-types found from breeding holdings were also detected from production holdings. As observed in breeding holdings, MRSA *spa*-type t127 was mainly isolated in Italian production holdings. Besides Italy, *spa*-type t127 was also isolated in one production holding in Cyprus and in two production holdings in Spain. MRSA *spa*-types non-ST-398 other than t127 were isolated in production holdings from four countries: mainly Italy and Germany, but also from one production holding in Spain and one in Norway.

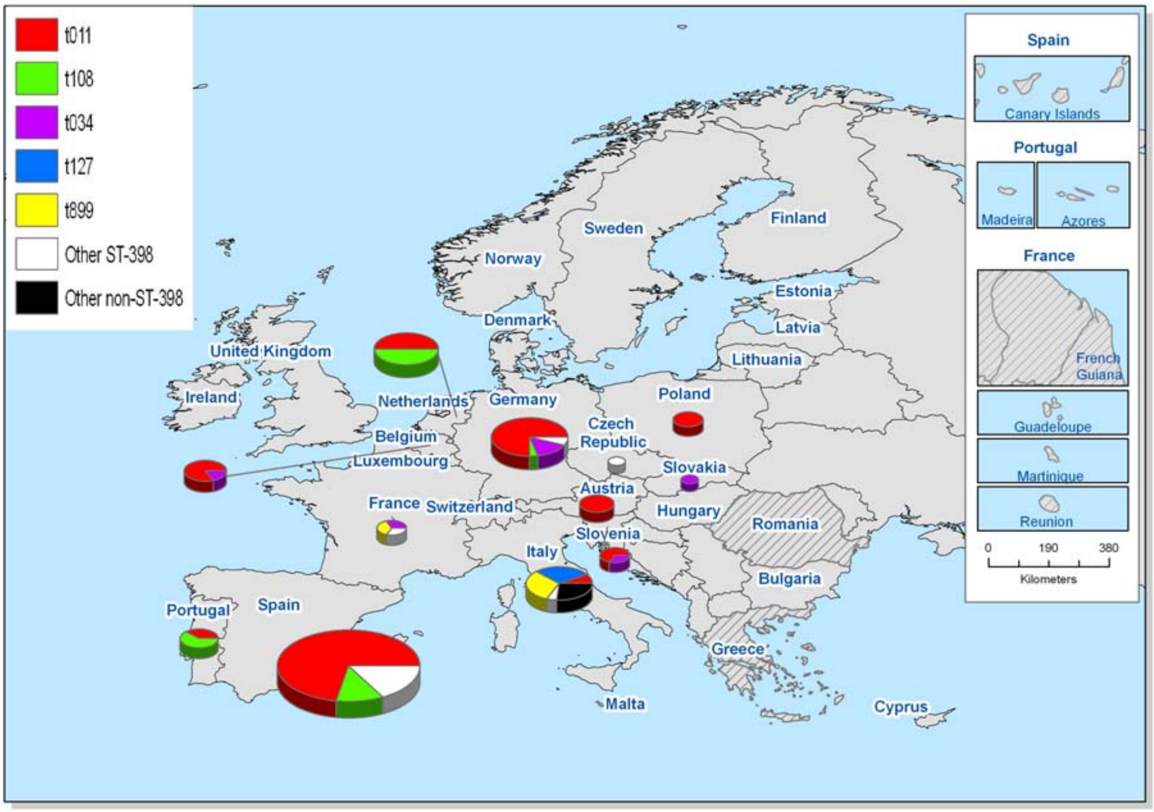

Figure 5: The distribution of *spa*-types of MRSA[(a)] in environmental dust samples collected from breeding pig holdings in the EU[(b)], Norway and Switzerland, 2008

(a): Pie chart sizes illustrate the number of MRSA isolates isolated in each country.

(b): Greece, Malta and Romania did not carry out the baseline survey: they are visualised on the map with dashed fill symbology.

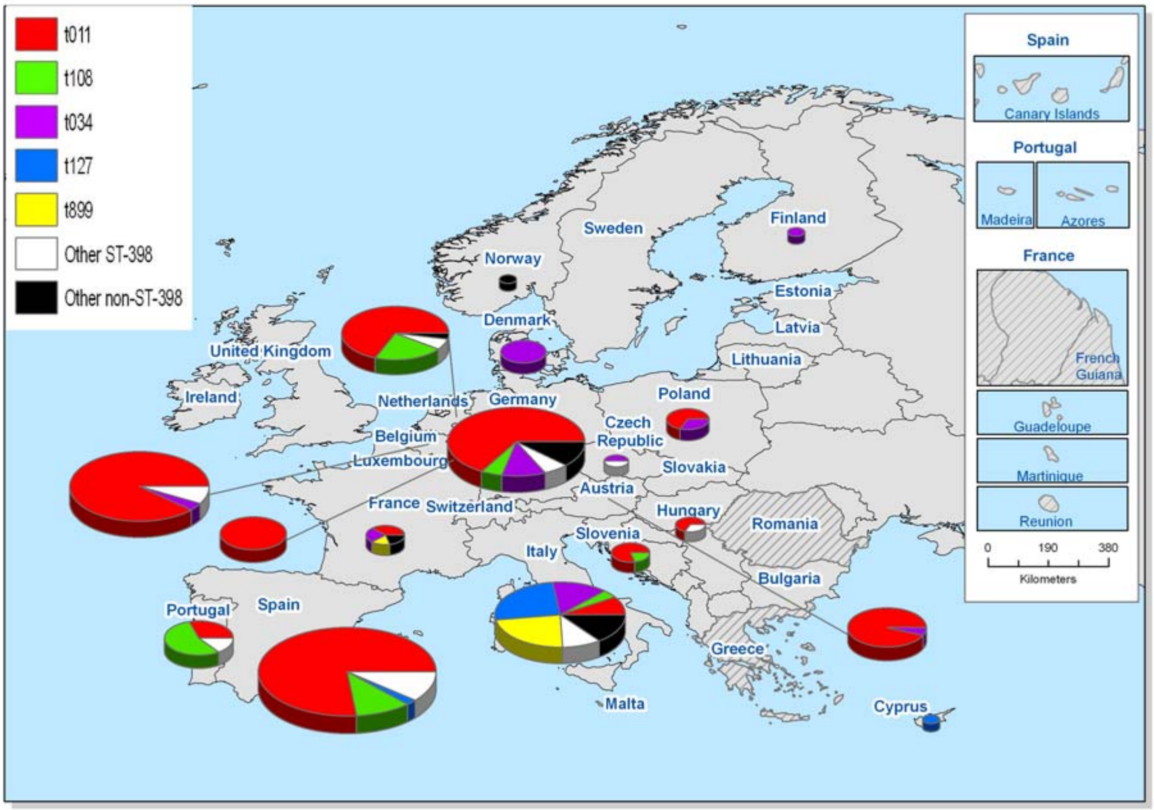

Figure 6: The distribution of *spa*-types of MRSA(a) in environmental dust samples collected from production holdings with breeding pigs in the EU(b), Norway and Switzerland, 2008

(a): Pie chart sizes illustrate the number of MRSA isolates isolated in each country. Note that, due to the large number of MRSA *spa*-types isolated in Spain, as only one *spa*-type t034 was isolated, the corresponding percentage represented in the pie chart is too small to be visible.

(b):Greece, Malta and Romania did not carry out the baseline survey: they are visualised on the map with dashed fill symbology.

4.4.2. PVL toxin analysis

Among the MRSA non-ST398 isolates found in the survey, 22 had *spa*-type t127 (MLST type ST1), one isolate had *spa*-type t008 (ST8) and one isolate had *spa*-type t002 (ST5). To investigate whether these 24 non-ST398 strains carried the genes involved in the production of the PVL toxin, they were subjected to PCR analysis specific to PVL-related genes (LukS-PV/LukP-PV). All 24 isolates tested negative by PCR analysis.

5. Discussion

The present report provides a further analysis of the dataset on MRSA in holdings with breeding pigs in the EU. This preliminary EU-wide survey estimated the prevalence of MRSA-positive breeding and production holdings across the EU, as previously described in the Part A report. Additional data collected by participating countries as part of the baseline survey was analysed to investigate factors associated with MRSA-positive holdings. Also, the association between the prevalence of MRSA-positive breeding holdings and the prevalence of MRSA-positive production holdings in a country was investigated. Moreover, country-level analyses were implemented using TRACES data providing preliminary insight on the association between the prevalence of MRSA-positive holdings with breeding pigs and the intra-Community trade in breeding pigs and the prevalence of MRSA-positive holdings. Lastly, the distribution of MRSA *spa*-types was analysed in detail.

5.1. Context of the MRSA baseline survey and study limitations

The setup of the survey on the prevalence of MRSA in breeding pigs was conditioned by the 2008 baseline survey on the prevalence of *Salmonella* in holdings with breeding pigs. The *Salmonella* survey was at an advanced planning stage when the MRSA survey was added to the scheme. Therefore, the survey design mainly focused on the investigations of factors related to the prevalence of *Salmonella*; and the pen, holding and management characteristics recorded were mainly related to putative risk factors for *Salmonella* contamination in holdings with breeding pigs. Linking both surveys had, however, 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 done during the visit for *Salmonella* sampling.

The *Salmonella* survey design aimed at investigating factors associated with *Salmonella* pen-level prevalence whereas the MRSA survey focused on holding–level prevalence and potential risk factors. This is the reason why environmental dust samples in the MRSA survey, collected from five different pens among the 10 sampled for *Salmonella*, were pooled prior to laboratory analysis and why no additional data identifying the five pens sampled for MRSA were collected. Consequently, no pen-level MRSA risk factor analysis could be conducted and potentially interesting data collected via the questionnaire pertaining to factors associated with *Salmonella* pen-level prevalence, such as antimicrobial usage or outdoor access, were of no benefit for the MRSA analysis.

Moreover, the *Salmonella* survey also afforded the opportunity to collect data on a few holding-level variables that might be associated with the MRSA status of the holding. MSs have their own characteristics for production and husbandry of breeding pigs, with differences in, for example, origin of replacement breeding stock, housing style, feed materials used, water quality and the potential for cross-contamination to other food production chains. While the *Salmonella* baseline survey attempted to record relevant data, many potential factors of relevance to *Salmonella* infection, such as specific sources of animals, feed and information on bio-security measures at holdings, were not part of the present survey. The pragmatic choice as to which potential risk factors to include or not was made by the MSs, partly based upon EFSA's proposal for the survey design (EFSA, 2007). Many potential factors of relevance to MRSA contamination, in particular those related to contacts of the holding with the outside, such as access to facilities and surroundings, specific sources of breeding pigs, number of suppliers of replacement gilts etc., were also not collected, although it has been recently postulated that transmission of MRSA between herds is associated with trade of carrier animals (Broens et al., 2009a).

As trade patterns of breeding pigs could not be attributed to individual holdings in this survey, and as information on trade of animals within countries was neither available, statistics available at MS-level on the intra-Community trade of breeding pigs from the EC were used to assess the potential association between the extent of the introduction of breeding pigs and the prevalence of MRSA-positive holdings in a complementary, ecological study.

5.2. Association between the prevalence of MRSA-positive breeding holdings and MRSA-positive production holdings

There was a strong association between the prevalence of MRSA and of MRSA ST398-positive breeding and production holdings in participating countries, and both statistical tests and complementary modelling confirmed this high correlation. However, because the modelling assumed that in each country only a sample of the existing holdings were investigated, while in reality in some countries a large fraction of all holdings were sampled, the estimate of the model-based correlation, which is close to 100%, is likely to overestimate the true correlation. The true correlation would still be higher than the value of the Spearman's correlation coefficient (76% for MRSA) but lower than 100%, assessed by the model used.

The relevance of MRSA contamination in breeding holdings is mainly related to the potential for vertical transmission of MRSA to production holdings through the supply of replacement breeding stock, mainly gilts. The significant correlation between the prevalence of MRSA-positive breeding holdings and the prevalence of MRSA-positive production holdings was consistent with the hypothesis of an epidemiological association between these two holding types within the same country. Should preventive and intervention measures be implemented, once identified and validated, both breeding and production holdings should be targeted. In particular, measures in breeding holdings would be important, because of the risk of vertical transmission.

Data exploring the reasons for this observed strong association between MRSA-positive breeding and production holdings were not collected in this survey, but the finding is in line with recently published studies. Intensive animal trade relations between farms of the same region, of the same pig primary production sector or of the same country probably contribute to the dissemination of specific bacteria in the pig population, once the bacteria have been introduced into the population. The importance of MRSA contaminated or colonised animals as vectors between farms has been pointed out (van Duijkeren et al., 2008). Vertical (top-down) transmission from nucleus to multiplier holdings and, further down the production pyramid, to production holdings has been found in the Netherlands (Broens et al., 2009a). Also, the co-existence of MRSA-positive and negative pig production chains within the same primary production sector was shown by Broens et al. (2009a). Actions to avoid the spreading of MRSA to countries, regions or holdings with lower MRSA prevalence may prove useful.

Higher similarities in MRSA *spa*-type isolates were pointed out by Tenhagen et al. (2009b) between slaughter pigs originating from the same region, as compared to ones from other regions, suggesting more intense relations and contacts between holdings from the same region through potential animate or inanimate vectors of MRSA. Broens et al. (2009b) suggested that lorries may be contaminated with MRSA and may contaminate pigs during transport, leading to further spread between farms whenever breeding animals are transported on contaminated lorries. To date, it is not known whether regional spreading of MRSA may occur via other animate and inanimate vectors, such as people moving from farm to farm, rodents or air-borne dust particles. The impact of these potential vectors on the spread of MRSA should be investigated in further studies.

5.3. Factors associated with MRSA contamination of breeding and production holdings

Holding-level information collected by participating countries as part of the baseline survey on *Salmonella* and MRSA in breeding pigs was further studied in the present report. Specifically, multivariable analyses were performed to explore the potential association between holding-level explanatory variables and MRSA contamination of holdings with breeding pigs. Analyses were carried out separately for breeding and production holdings, including both MSs and non-MSs participating in the survey. As no important differences were observed between the identified factors associated with MRSA or with MRSA ST398 contamination, it was decided to only report the results of the analyses of factors associated with the detection of MRSA. Moreover, as the specific prevalence of MRSA non-ST398 positive holdings was low in the EU (EFSA, 2009b), risk factor analyses for this small number of cases would have been neither feasible (lack of statistical power) nor meaningful, and was therefore not undertaken in the present report.

Of the potential risk factors analysed at EU-level only holding size showed a statistically significant association with the risk of MRSA contamination. Both breeding and production holdings with a higher number of breeding pigs were at a higher risk of being MRSA-contaminated compared to holdings having a smaller adult herd size. A breeding holding and a production holding housing respectively more than 400 and 100 breeding pigs were twice as likely to be MRSA-contaminated compared to breeding and production holdings with less than 100 breeding pigs. Holding or herd size is frequently investigated and detected as a risk factor for pig diseases or infections. Biologically plausible reasons for a positive association between holding size and infection include a greater risk of the introduction of infectious agents from outside the holding, a greater risk of transmission of agents within and among holdings when the holding is large, and effects of management and environmental factors that are related to holding size (Gardner et al., 2002). Larger holdings with breeding pigs probably need important genetic input resulting in more trading contacts and purchases of replacement breeding stock, which may put them at a higher risk of introduction of agents of either animal or public health significance. Similarly, within-herd circulation of pathogens is more likely in larger holdings, as more potential shedders and recipients of the pathogen are present. Furthermore, holding size might be a marker of risk related to a number of underlying risk factors that contribute to the holding size effect (Wagenaar and van de Giessen, 2009), which still need to be investigated. The herd size effect is consistent with the results of recent studies. In the Netherlands, a study based on data from this baseline survey and on additional national data (Wagenaar and van de Giessen, 2009) also reported holding size as the factor most strongly related to MRSA holding contamination. Similarly, in Germany and Italy, herd size has been also identified as one factor related to the risk of MRSA colonisation in fattening pig herds (Tenhagen et al., 2009a; Battisti et al. 2009).

The factor “gilt replacement policy” was weakly associated with the prevalence of MRSA-positive breeding holdings in this study, and did not remain in the final model. However, this indicated that breeding holdings with more than 90% of their gilts purchased tended to have a higher prevalence compared to breeding holdings with more than 90% of their gilts home-bred and consequently that buying replacement gilts from other farms (whatever the MRSA status of the holding of origin) was weakly associated with the risk of introducing MRSA into breeding holdings. To date, MRSA was not included in biosecurity protocols of pig holdings because it was not considered a relevant disease agent (van Duijkeren et al., 2007; Meemken et al., 2009). Therefore, no measures to control replacement stock for MRSA were taken and this may have facilitated the spread of MRSA into nucleus and breeding herds.

A second factor that importantly influenced the risk of MRSA-contaminated breeding and/or production holdings was the country, because the country random effect was significant, in both models. About two-thirds of the total variance in MRSA prevalence could be attributed to country in the breeding holding as well as in the production holding models. This can be explained by the considerable variation in MRSA prevalence that was observed among countries (EFSA, 2009b). The survey results showed that there were countries with a high prevalence as well as countries with low numbers of, or no, MRSA-positive holdings detected.

As explained before, the present report did not aspire towards a comprehensive analysis of all possible factors generally believed to impact on the risk of breeding pig holdings becoming contaminated with MRSA. Instead the aim was to assess those few holding-level factors for which information was captured by a questionnaire used in this survey. In some instances the information analysed pertained to husbandry methods and type of holdings. Such factors may reasonably be regarded as potential risk factors. However, in this survey, no significant association was found between the factors ‘type of breeding or production holdings’ and MRSA-positivity of the holding. In other instances the information pertained to the sampling and testing protocol, notably to the delay between the sampling date and the testing date at the laboratory. Whilst not strictly a risk factor, this factor may have had an impact on the results, but the analyses showed that it was not associated with MRSA-positivity of the samples in this survey. Furthermore, the analyses revealed that there were no significant differences in the likelihood of holdings to be tested MRSA-positive in the different quarters of the year (time of sampling during the year).

5.4. Association between intra-Community trade in breeding pigs and prevalence of MRSA-positive holdings

At country-level, the prevalence of MRSA was strongly associated with volumes of imported breeding pigs into the countries, such as numbers and proportions of imported breeding pigs, numbers of imported consignments or numbers of trading partners (countries of origin). This was, in particular, the case for the numbers of breeding pigs when the imported breeding pigs originated from countries where the pigs were at risk of being MRSA contaminated, i.e. from countries having higher MRSA holding prevalence. This was analysed by using a proxy for number of imported breeding pigs at risk of being contaminated with MRSA. This is, on an international scale, in line with the observation that fattening pig units buying pigs from more than one source had substantially higher prevalence rates than fattening units in farrow-to-finish operations that did not purchase pigs (Denis et al., 2007; Tenhagen et al., 2009a).

The association between the import volume of breeding pigs and the prevalence of MRSA-positive breeding holdings in the country was stronger compared to the disclosed association with production holdings. Although TRACES data did not identify the level of the production pyramid that the pigs originated from, it is likely that genetically more valuable animals intended for breeding holdings could have been transported over longer distances, while gilts for production herds are more likely to originate from local production.

The role and intensity of animal movements in the spread of infectious diseases is well established (Gilbert et al., 2005; Fevre et al., 2006). Until recently, MRSA were not in the focus of any biosecurity protocols because *S. aureus* was not considered an important pathogen of animal health in pigs, and the zoonotic potential of *S. aureus* from pigs was unknown. Hence, *S. aureus* was not included in sampling protocols for pigs prior to trading or in quarantine as addressed in Section 5 of the Terrestrial animal health code of the Office International des Epizooties (OIE, 2009). In light of the findings of the current survey, intervention studies to assess the effectiveness of checking the MRSA status of replacement breeding pigs as a preventive measure of MRSA diffusion would seem to be justified.

5.5. Analysis of the MRSA *spa*-types distribution across the EU

5.5.1. Spatial distribution of MRSA *spa*-types

Spa-typing is an excellent tool for national and international surveillance of *S. aureus*, particularly in combination with other markers.

The general distribution of *spa*-types and possible associations with reported *spa*-types in scientific and published literature were described in the Part A report (EFSA, 2009b). The analyses of this Part B report indicated that the distribution of *spa*-types is significantly different between countries. Particularly Italy had a specific *spa*-type distribution of its own. Most *spa*-types isolated from breeding holdings were also detected in production holdings within the same country.

When comparing the results of this survey with the published distributions of *spa*-types, the following observations can be made. Denis et al. (2009) reported that two genotypes of MRSA ST398 were predominant in pigs and pig farmers in Belgium, t011-SCC*mec*IVa and t011-SCC*mec*V. Among humans in contact with pigs they also detected t034-SCC*mec*V and t567. These results are in line with the Belgian survey results as part of the EU survey where the majority of the MRSA isolates were *spa*-type t011, and two were t034 and t567. Denis et al. (2009) showed that resistance patterns were related to clonal types. The relation between resistance patterns and genotype has not yet been investigated for the isolates recovered during this survey, as the reported results of susceptibility testing were too scarce to be analysed. The Belgian study also illustrates that MRSA isolates with the same *spa*-type may possess a different genotype. The observation that MRSA isolates with the same *spa*-type can have a different genotype suggests that further investigation of the isolates recovered in this survey is required before the geographical distribution of isolates of different genotype can be fully investigated and to verify whether particular patterns of antimicrobial resistance are associated with particular genotypes. The presence of isolates of the same *spa*-type with a different genotype was also observed in a study involving pigs at several abattoirs in Germany (Tenhagen et al. 2009b). The three predominant *spa*-types detected in the EU survey in Germany were the same as those detected by Tenhagen et al. However, the most common *spa-type*s t011 and t034 could be subdivided by SCC*mec* type into different genotypes by Tenhagen et al.

The possibility that pig herds positive for one MRSA lineage might contain other MRSA lineages (possibly at a lower prevalence) was not addressed by the survey protocol.

In Italy, where a specific *spa*-type distribution in the survey occurred, the most prevalent *spa*-type was t899 (in ST398 group) and related *spa*-types, both in breeding and production and in fattening pig holdings (Battisti et al., 2009). This *spa*-type had been described for the first time in different kinds of (farrowing and finishing) piggeries in the Netherlands (van Duijkeren et al., 2008), although during the MRSA baseline survey no such *spa*-type was detected from Dutch breeding and/or production holdings. Additionally, non-ST398 *spa*-types, like t127 (ST1), t1730 (ST1476: CC97) were represented among isolates from Italian breeding, production and fattening holdings, demonstrating a wider heterogeneity of clones circulating in this country. These features suggest that other sources, besides trade contacts, and other factors may be involved in spreading within and among holdings of a given country, after the introduction of certain MRSA clones.

5.5.2. PVL toxin analysis

MRSA clones have emerged independently in at least three separate settings: human hospitals (HA-MRSA), human carriers outside of hospitals (community) (CA-MRSA) and livestock (LA-MRSA). MRSA may also be commonly isolated from companion animals (EFSA, 2009a). MRSA ST398 comprises a further subgroup of MRSA, associated with pigs, which for certain professional groups (e.g. pig holders and their families, veterinarians) might be considered an occupational health risk.

CA-MRSA and HA-MRSA tend to differ at molecular level, and thus, these genetic differences can be used to investigate whether isolates show the general characteristics of CA-MRSA or HA-MRSA. Assigning the isolates to one or other category is likely to be important for assessing potential public health significance. CA-MRSA often produce PVL, especially in the case of isolates originating from the North American continent. PVL is epidemiologically linked to the five predominant CA-MRSA clonal lineages widely associated with large disease outbreaks (Diep and Otto, 2008); it can also be found in sporadic CA-MRSA and methicillin susceptible *S. aureus* (MSSA) strains. In animal-associated MRSA, production of the PVL toxin is rarely seen (Kadlec et al., 2009, Walther et al., 2008); though the toxin has been detected in isolates from companion animals in an American study (Rankin et al., 2005).

In this EU-survey, MRSA ST398 isolates were not tested for PVL genes because isolates of this organism recovered from European pigs in previous national studies, have consistently proved negative for PVL, when they have been tested. It is possible for *S. aureus* ST398 to acquire PVL and there is a recent report of an isolate of a MSSA ST398 with PVL from a single case of human illness in France (Laurent et al., 2009). These authors comment on the difference observed between porcine MRSA ST398 and human MSSA ST398 with respect to possession of PVL toxin.

Still, a minor subset of MRSA isolates recovered in the current EU survey belonged to STs not previously found among pigs (ST1, ST5 and ST8). *Spa*-typing and the PVL toxin status were used by EU-RL to investigate and classify further these isolates, in order to assess better possible public and animal health significance. *S. aureus* belonging to ST1, ST5 and ST8 are frequently seen among CA-MRSA isolates in humans. Differences have been observed in the geographical origin of human isolate and PVL status. Isolates of ST1, ST5 and ST8 from the EU are often PVL-negative. However, in the USA, USA300 and USA400 CA-MRSA clones have been detected that belong to ST8 and ST1, respectively, and that are PVL-positive (Vignaroli et al., 2009). Some European countries have reported the presence of single cases or clusters of PVL-positive USA300, ST8 infections in man, but the predominant CA-MRSA lineage in humans in Europe is reported to be ST80 (Deurenberg et al., 2007). USA400 is reported to be rare in Europe (Vignaroli et al., 2009). Nevertheless, t127, ST1 MRSA IVa, PVL-negative, appears to be a common cause of CA-MRSA infection in the United Kingdom, often associated with drug users and homeless people (Otter et. al, 2009).

USA400/ST1 isolates generally belong to *spa*-type t128, a different *spa*-type from that recorded in pigs in this survey (t127), while USA300/ST8 isolates are *spa*-type t008. The ST8 isolates detected in this survey were also *spa*-type t008; however, the PVL-negative status of the isolates detected in pigs suggests that they are different from the CA-MRSA strains occurring in the USA and probably relate to MRSA strains currently circulating in humans in the EU.

As these STs are commonly found among CA-MRSA in humans, their presence in pigs could indicate that the isolates of non-ST398 MRSA originate from humans. CA-MRSA from humans can produce the PVL toxin encoded by the LukS-PV/LukP-PV genes, however, exceptions do occur (Diep and Otto, 2008). In contrast to this, MRSA isolates from livestock animals rarely carry the LukS-PV/LukP-PV genes (Kadlec et al., 2009; Smith et al., 2009; Walther et al., 2008), including t127, ST1 MRSA from fattening pig holdings (Battisti et al., 2009). Therefore, further molecular typing methods, such as Pulsed Field Gel Electrophoresis (PFGE) and Staphylococcal cassette chromosome mec (SCCmec) typing, of these isolates as well as selected human isolates of the same STs would be required in order to establish stronger evidence of human-to-porcine transfer rather than the opposite. In the case of the single t008 (CC8) isolate from Norway, such a link has in fact been shown recently (Sunde et al., 2009).

The molecular work performed on these isolates by EU-RL, in particular the absence of the PVL toxin in ST1 and ST8 therefore suggests that: (1) the ST1 and ST8 isolates are not related to the PVL-positive, CA-MRSA strains present in the USA and designated USA300 and USA400; (2) the isolates could be related to MRSA isolates currently present in humans in the EU; however, further typing is required in order to support this further.

5.6. Importance of the findings for MRSA diffusion among pig holdings in the EU

It may be assumed that the risk of MRSA colonisation and contamination among pigs results from two different components, the risk of introduction into and the risk of dissemination within the population of animals/holdings. The findings of this report are in line with this concept. At country level, there was a strong association between MRSA prevalence and the volume of imports into the country of breeding pigs at risk of MRSA colonisation. At pig primary production sector level, the strong association observed between the prevalence of MRSA-positive breeding holdings and the prevalence of MRSA-positive production holdings is likely to reflect vertical (top-down) diffusion along contaminated pig primary production chains. At holding level, the holding size factor may reflect, among others, the negative impact of more numerous contacts in larger holdings, increasing the risk of contact with MRSA positive sources, mainly through the introduction of replacement breeding pigs, and/or other vectors, e.g. rodents (van de Giessen et al., 2009).

Further studies are needed to look at risk factors for the introduction, spread and persistence of MRSA in the country, in integrated pig primary production chains and on the holdings, preferentially at MS level, since epidemiological situations seem to differ importantly among countries. Such surveys should focus on the origin, holding(s) and country(ies), of the replacement breeding pigs purchased and to address the trading flows considering the epidemiological situations and status of the sources of animals regarding MRSA. The use of antimicrobial agents during the production cycle should also be investigated.

As common types of MRSA (in particular MRSA non-ST398) may colonise both pigs and humans, the risk of transmission from humans to pigs as well as the risk of colonised humans providing an initial source of introduction of MRSA to pig herds free of colonisation should be addressed. The potential history in the recent past of reported MRSA cases in working personnel (farmers, workers, veterinarians), of hospitalisation (e.g. in intensive-care units) and of any frequent contact made with health-care workers, physicians etc. could be gathered and addressed as part of such investigations.

It may be assumed that countries with a high MRSA prevalence in breeding and production holdings would also have a high prevalence of MRSA in the fattening pig population. This would mean a potentially important occupational exposure of livestock and slaughterhouse professionals to MRSA and the subsequent risk of introduction of MRSA from pig origin, MRSA ST398 or MRSA non-ST398, into the community, i.e. in the human population not in direct contact with pigs or carcasses, and the health-care facilities via those exposed. Moreover, as shown recently in the Netherlands (de Boer et al. 2009), raw meats, including pork, may be contaminated with MRSA, although the prevalence of MRSA in meat has not been shown to contribute significantly to the dissemination of MRSA to humans (EFSA, 2009a). Further assessment of the public health significance of MRSA from pig origin, besides potential infections of occupational character, is therefore necessary, in particular for MRSA non-ST398. The spread of MRSA of pig origin, MRSA ST398 and MRSA non-ST398, should also be assessed further in the community.

In this preliminary survey, MRSA types other than the MLST-type ST398 were observed. The sources of MRSA in the pig population, of the emergence and of the spreading of new clones, that might present particular pathogenic features, must consequently be investigated. It would be advisable to routinely characterise MRSA isolates derived from pigs by using molecular typing methods in order to facilitate timely detection of changes in the MRSA population. Thus far, MRSA is assumed to be transmitted from pigs to humans; however, the opposite direction may also play a role. Monitoring of the occurrence and clonal diversity of MRSA in pigs, as well as in other relevant animal species, and meat thereof, should therefore be performed in parallel with that carried out in the human community, to investigate further the transmission of MRSA between animals and humans and enable the detection of the spreading of new clones.

CONCLUSIONS

This report provides for the results from further analyses of the MRSA baseline survey in holdings with breeding pigs in the EU. The factors potentially associated with MRSA-positive holdings, the intra-Community trade of breeding pigs, the distribution of MRSA *spa*-types across MSs and the PVL toxin production of some MRSA isolates were investigated in order to improve the knowledge of the epidemiology of MRSA in pigs across the EU.

- In breeding as well as in production holdings, a formal multiple regression analysis showed that the risk of contamination with MRSA increased with increasing numbers of breeding pigs in the holding (larger holding size). A breeding holding and a production holding housing respectively more than 400 and 100 breeding pigs were twice as likely to be contaminated with MRSA compared to breeding and production holdings with less than 100 breeding pigs. The holding size effect may reflect a greater risk of introduction and of within-holding diffusion of MRSA in larger holdings, notably through a more intensive introduction of replacement breeding animals, but also the impact of other unmeasured underlying risk factors that are associated with structural characteristics and/or managerial practices typical of larger holdings.
- The risk of contamination with MRSA for breeding and for production holdings varied significantly between countries, even when adjusting for the effect of the holding size.
- There was a strong association between the prevalence of MRSA-positive breeding holdings and the prevalence of MRSA-positive production holdings in countries suggesting vertical transmission of MRSA from breeding holdings to production holdings.
- In this survey, no significant association was found at EU level between the prevalence of the MRSA-positive holdings and the type of breeding or production holding, the gilt and boar replacement policies, the delay between the sampling date and the testing date at the laboratory, and the quarter of sampling during the year 2008.
- More detailed information on several factors associated with bio-security, at holding level, as well as information on a number of factors such as those related to rearing or usage of antimicrobial agents, were not investigated in the survey. Therefore, it was not possible to estimate the association of these factors with MRSA contamination of holdings and their potential confounding role with factors for which data were available. However, results of this analysis are useful starting points for more specifically aimed studies in individual countries.
- A complementary descriptive analysis using TRACES (TRAde Control and Expert System of the Community) data on intra-Community trade disclosed a strong positive association between the prevalence of MRSA-positive holdings with breeding pigs and the volume of imported breeding pigs. The association was particularly strong when the breeding pigs originated from countries having higher MRSA holding prevalence (with the proxy-number of breeding pigs at risk of being contaminated with MRSA).
- The *spa*-type distribution of MRSA in breeding and production holdings differed significantly between countries. One MS in particular had a specific *spa*-type distribution compared to other participating countries.
- No PVL genes were detected in the ST1, ST5, and ST8 isolates from pigs recovered in the framework of the baseline survey in the EU, indicating that those strains are not likely to be related to PVL-positive, CA-MRSA strains prevalent among humans in other parts of the world.

RECOMMENDATIONS

- It would be useful to carry out further national studies to identify more closely the factors that put holdings with breeding pigs at risk of MRSA contamination in the country in order to complement the findings of this survey.
- Investigations on the impact of addressing MRSA in on-farm biosecurity and managerial practices are needed as a basis for the development of potential future preventive and intervention measures to contain MRSA contamination of pig holdings. In particular, it would be important to investigate measures to prevent the introduction of MRSA into MRSA-negative holdings. As a starting point, the checking of the MRSA status of the replacement breeding pigs could be specifically considered.
- It is advisable to characterise MRSA isolates derived from pigs by using molecular typing methods to facilitate the detection of the emergence and spreading of new clones in the MRSA population.

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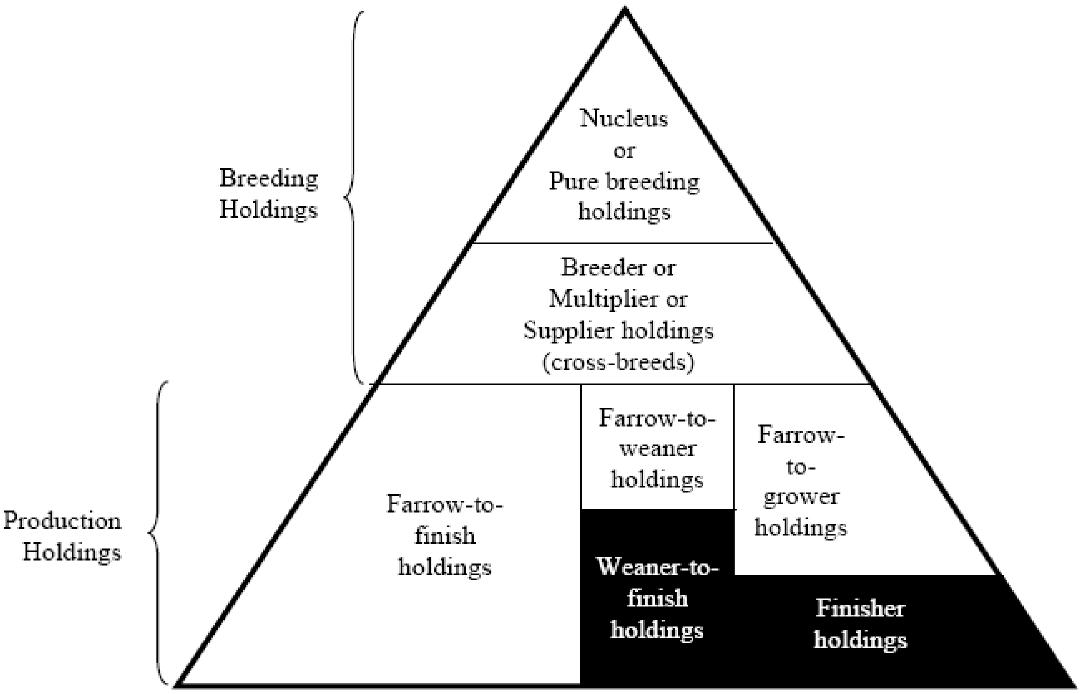
APPENDICES

TABLE OF APPENDICES

A. 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 SURVEY[13]



At the top in the pig primary production pyramid are "*pure breeding, or nucleus, herds"* that follow special selection procedures and deliver boars for production of semen at boar stations. These herds can also deliver purebred boars and gilts to all other 'production holdings (farrow-to-finish, farrow-to-weaner and farrow-to-grower holdings)'.

Beneath this '*pure breeding*' there are "*multiplier herds*". These herds deliver replacement breeding pigs, mainly sows, to all production herds.

The latter production holdings, often referred to as "*commercial, or piglet-producing herds*", produce piglets and keep them until weaning (farrow-to-weaner), until the first stage of fattening (farrow-to-grower) or covering the whole production phase (farrow-to-finish). The latter and the weaner-to-finish holdings as well as the finisher holdings produce pigs typically called slaughter pigs and are sent to slaughter at the end of the growing/finishing period. Some of the piglet producing herds, can also have units for slaughter pigs, and are usually called "*farrow-to-finish herds*".

Weaner-to-finish and finisher holdings are not covered by the MRSA survey.

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[13] Please refer to Glossary at end of report for definitions.

B. POTENTIAL ASSOCIATED FACTORS INVESTIGATED IN THE SURVEY AND CATEGORISATION USED IN THE ANALYSIS

Variable	**Categories used**
Country	Country
Testing delay (in days)	0-1 2-4 5-7 >7
Time of sampling	January-March April-June July-September October-December
Type of breeding holdings	Nucleus Multiplier or supplier
Type of production holding	Farrow-to-weaner Farrow-to-grower Farrow-to-finish
Size of the holding (in number of breeding pigs)	<100 100-499 500-999 >999
Gilt replacement policy	>90% home-bred 10-90% home-bred >90% purchased
Boar replacement policy	No boars on farm >90% home-bred 10-90% home-bred >90% purchased

C. OVERVIEW OF THE OPTIONAL VARIABLES REPORTED IN THE FRAMEWORK OF THE BASELINE SURVEY[(a)]

Country	**No holdings sampled**	**Location of the holding**		**MLST typing**		**Result of SCCmec typing (main group)**		**Result of SCCmec typing (subgroup)**		**Result of antimicrobial susceptibility testing**	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Austria	242	242	100								
Belgium	199	199	100	3	1.5						
Bulgaria	72	67	93.1								
Cyprus	65										
Czech Republic	267	262	98.1	3	1.1	3	1.1				
Denmark	293	7	2.4	6	2.0						
Estonia	34	34	100								
Finland	198			1	0.5	1	0.5				
France	342										
Germany	201			8	4.0						
Hungary	181	181	100	3	1.7	3	1.7	2	1.1	3	1.7
Ireland	189										
Italy	214	214	100								
Latvia	33	33	100								
Lithuania	82	82	100								
Luxembourg	44	44	100	2	4.5	2	4.5	2	4.5	4	9.1
Poland	321	320	99.7	8	2.5	8	2.5				
Portugal	170	170	100								
Slovakia	192	190	99	1	0.5						
Slovenia	114	111	97.4	8	7.0						
Spain	359										
Sweden	202	194	96								
Netherlands	321	321	100								
United Kingdom	258	258	100								
EU Total	**4,593**	**2,929**	**63.8**	**43**	**0.9**	**17**	**0.4**	**4**	**0.1**	**7**	**0.1**
Norway	251	251	100	1	0.4	1	0.4	1	0.4	1	0.4
Switzerland	225	224	99.6								

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

D. STATISTICAL METHODS USED IN THE RISK FACTOR ANALYSIS

Collinearity diagnostics

As collinearity diagnostics both VIF and the condition index were calculated (using SAS PROC REG), both including and excluding country as a variable.

The VIF is an index that reflects how much the variance of an estimated regression coefficient is increased because of collinearity, compared to a situation were no correlation between independent variables is present (as in a completely balanced study design). The VIF for a particular regression coefficient is calculated by regressing the variable on all other independent variables in the model. The VIF is then calculated as $1/(1-R^2)$, where R^2 is the percentage of variance explained by this model. Values greater than 10 are seen as indicating the presence of serious collinearity. The condition index is another measurement reflecting that high values may indicate that the inversion of the covariance matrix of the independent variables (part of the regression procedure) is numerically unstable.

There were no indications that multicollinearity is present in this analysis: all VIF values were below 10, and the condition index was below 30.

Statistical models fitted

As only 41 cases of MRSA non-ST-398 are present (nine in breeding holdings, 33 in production holdings), the statistical power was too low for a sensible analysis of specific risk factors for the contamination with this subgroup of MRSA.

For all MRSA, the general rule of thumb for logistic regression models is that the number of cases should be at least 10 times the degrees of freedom of the model, was used (Hosmer and Lemeshow, 2000). For models in which country is used as a random intercept, one degree of freedom is needed for including country as a random effect, while 25 degrees of freedom are needed for including country as a fixed effect.

With 145 cases of MRSA in breeding holdings and 417 in production holdings, using country as a fixed effect is not possible for breeding holdings. Moreover, in random effect models, countries with zero prevalence can be included in the analysis, while this is not possible in fixed effect models as this is causing quasi-complete separation of data.

Therefore multivariable logistic models were fitted using country as a random intercept, using full integration of the random effects (SAS PROC GLIMMIX with Gaussian quadrature). Such a model assumes that the prevalence rates from countries are normally distributed on the log-odds scale. As there is a wide range of prevalence rates between countries, this assumption is probably violated in this case. Therefore, sandwich estimates (option EMPIRICAL) were used for calculating CIs of the odds ratios from these models, as these are robust towards misspecification of the model. The bias correction suggested by Morel, Bokossa, and Neerchal (2003), (option EMPIRICAL=MBN) which is needed when the number of samples (here: countries) is low, was used. Using the (bias corrected) sandwich estimator protects against drawing conclusions that are artefacts from the assumptions in the model, rather than from the data. However, the price to be paid for this is that some power is lost.

Using random effect models with country, all variables could be entered simultaneously for production holdings for all MRSA and MRSA ST-398. For breeding holdings, a full model does not quite meet the requirements of 10 cases per degree of freedom. However, when grouping the variable “Month of the Year” into four quarters, and “testing delay” into four groups, the model degrees of freedom is 18, and, although not completely complying with the general rule, it is close enough to be fitted without overfitting the data.

In order to be consistent, this same grouping of month and delay time was used for all other multivariable analyses. For all MRSA and MRSA ST-398 in production holdings, models using country as fixed effect (excluding countries with zero prevalence) are also possible. These models were also fitted, and gave similar results to the random effect models that are shown here.

Furthermore, for each variable, the interaction term with production type (breeding or production holding) was tested for statistical significance (using a likelihood ratio test) using all data and a full model including all risk factors. This interaction did not reach statistical significance for any of the variables. Therefore, analyses combining breeding and production holdings were also carried out.

Finally, a back step procedure was used, in which the variable with the highest (type 3) *P*-value was removed until only variables remained in the model with a *P*-value <0.05.

Country-specific analysis

To prevent false positive finding from doing too many statistical analyses, country-specific analyses were only carried out when a statistically significant interaction was present between a variable and a country. The models containing interaction terms were fitted in the group of countries with more than 30 cases. This interaction was only statistically significant (*P*=0.014) for season. Further country-specific analyses for this variable showed that this risk factor was only statistically significant in Belgium, showing a decrease in MRSA prevalence over the year (odds ratio: April-June: 0.408 [0.123; 1.354]; July-September: 0.375 [0.113; 1.241]; October-December: 0.349 [0.110; 1.102]).

Analyses of interactions

The presence of interaction between holding size and all other risk factors and between the variables: gilt replacement policy and boar replacement policy, was further checked. Holding size was used because in all analyses in production holdings this was a strong risk factor for MRSA prevalence. None of the interactions between holding size and the other variables were statistically significant, only the interaction between gilt replacement policy and boar replacement policy was borderline statistically significant for ST-398 (*P*=0.07). This was mostly due to the category “no boar on the farm, more than 90% of gilts home-bred”, which had a higher prevalence of MRSA ST-398 than expected from the separate variables “boar replacement policy” and “gilt replacement policy”.

Therefore, also an indicator variable “closed-no boar” was added to all random effect models. No statistically significant effects were seen, so this variable was not added to any of the final models.

E. CORRELATION BETWEEN MRSA PREVALENCE IN BREEDING AND IN PRODUCTION HOLDINGS

- Additional scatter diagrams

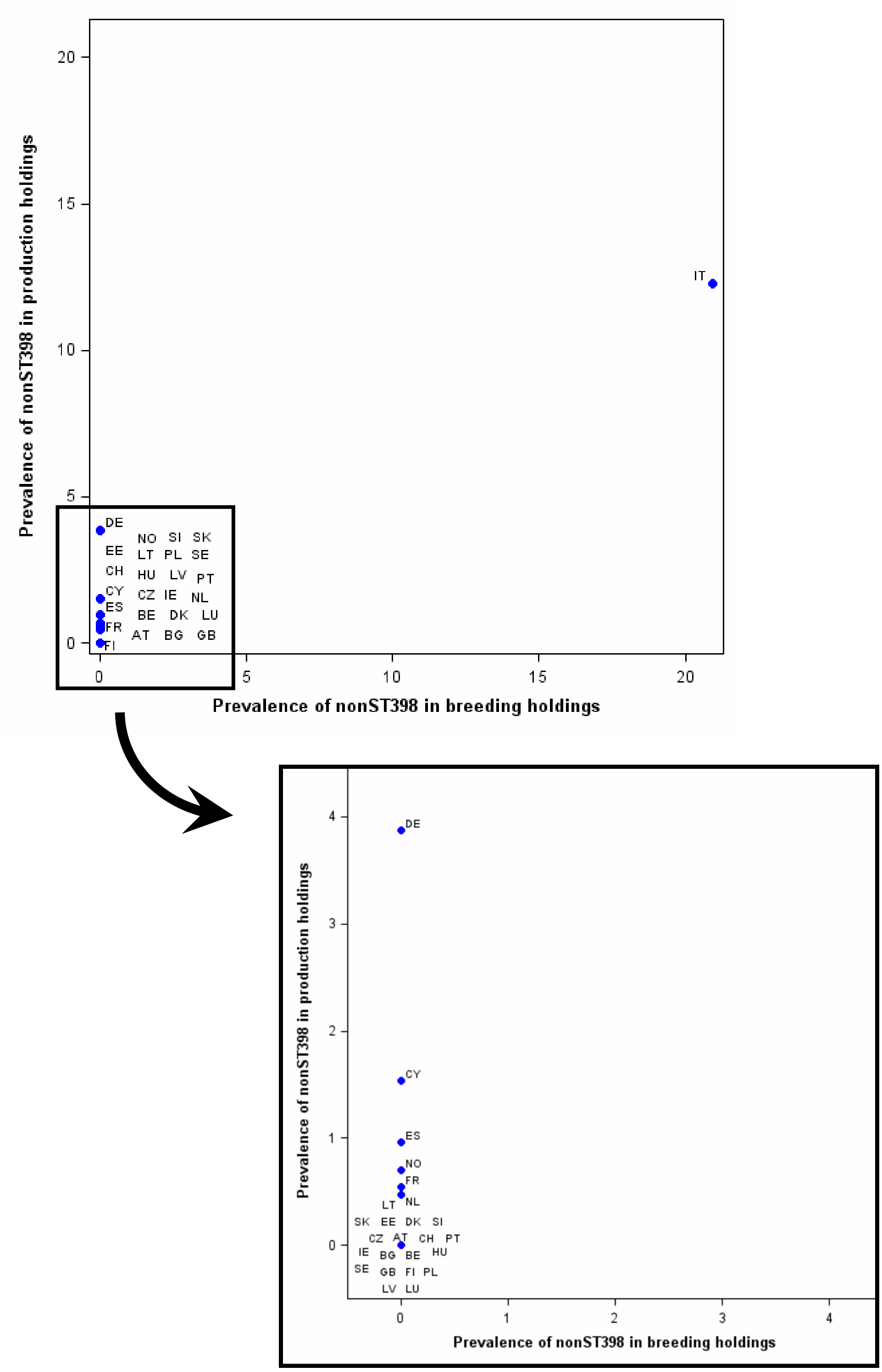


Figure 7: Scatter diagram of MRSA non-ST-398 prevalence in breeding holdings vs. MRSA non-ST-398 prevalence in production holdings, MRSA EU baseline survey in breeding pigs, 2008 (a)

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

- Correlation estimates by modelling

In addition to calculating correlation coefficients between MRSA prevalence in breeding holdings and in production holdings, a model was also built in which the observed prevalence was assumed to be a realisation of an underlying real prevalence rate. The model was a multivariate logistic random effect model. This model is multivariate in the sense that it has a multivariate outcome, consisting of both prevalence in production holdings and prevalence in breeding holdings. The only fixed effect in this model was production type. The random effect in this model consists of separate random country effects for production and breeding holdings, and their covariance. In this model it is possible to calculate the correlation between the random effects of both production types throughout the countries. The significance of this correlation was determined by testing the significance of having a covariance term between the random effects for production and breeding holdings using a likelihood ratio test.

The correlation estimated with this model was >0.99, indicating that the lower correlation coefficients of the observed data could be fully explained by sampling variability.

Table 5: Correlation between the MRSA/MRSA ST398/MRSA non-ST398 prevalence in breeding holdings and in production holdings. MRSA EU baseline survey in breeding pigs, 2008

Prevalence	**Correlation of random country effects**	***P*-value**
MRSA[a]	>0.99	<.0001
MRSA ST398	>0.99	<.0001

(a): all *spa*-types

F. DESCRIPTIVE ANALYSIS OF FACTORS POTENTIALLY ASSOCIATED WITH MRSA POSITIVITY IN BREEDING AND IN PRODUCTION HOLDINGS

Table 6: Prevalence of breeding holdings tested positive for MRSA/ST398/non-ST398 by potential associated factor, MRSA baseline survey in breeding pigs, 2008 [(a)]

	All MRSA			ST398		Non-ST398	
	N	**N pos.**	**prev.**	**N pos.**	**prev.**	**N pos.**	**prev.**
Testing delay (in days)							
≤1	544	29	5.3%	29	5.3%	0	0.0%
2-4	301	14	4.7%	11	3.7%	2	0.7%
5-7	440	19	4.3%	17	3.9%	2	0.5%
>7	315	83	26.3%	78	24.8%	5	1.6%
Quarter of sampling							
January-March	303	25	8.3%	25	8.3%	0	0.0%
April-June	451	38	8.4%	34	7.5%	3	0.7%
July-September	370	31	8.4%	27	7.3%	4	1.1%
October-December	476	51	10.7%	49	10.3%	2	0.4%
Holding type							
Multiplier	1200	115	9.6%	105	8.8%	9	0.8%
Nucleus	400	30	7.5%	30	7.5%	0	0.0%
Holding size (in number of breeding pigs)							
<100	373	10	2.7%	9	2.4%	1	0.3%
100-399	732	62	8.5%	60	8.2%	2	0.3%
400-999	323	45	13.9%	42	13.0%	2	0.6%
>999	172	28	16.3%	24	14.0%	4	2.3%
Gilt replacement policy							
>90% gilts home-bred	983	94	9.6%	92	9.4%	2	0.2%
10-90% gilts home-bred	197	12	6.1%	10	5.1%	2	1.0%
>90% gilts purchased	420	39	9.3%	33	7.9%	5	1.2%
Boar replacement policy							
no boars on farm	188	16	8.5%	15	8.0%	1	0.5%
>90% home-bred	630	75	11.9%	70	11.1%	4	0.6%
10-90% purchased	114	10	8.8%	9	7.9%	1	0.9%
>90% purchased	668	44	6.6%	41	6.1%	3	0.4%

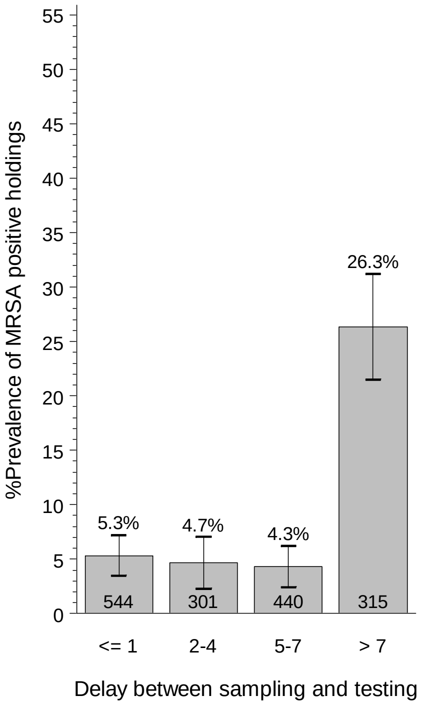
(a): Greece, Malta and Romania did not conduct the survey and two non-MSs, Norway and Switzerland, participated.

Table 7: Prevalence of production holdings tested positive for MRSA/ST398/non-ST398 by potential associated factor, MRSA baseline survey in breeding pigs, 2008[(a)]

	All MRSA			ST-398		not ST-398	
	N	N pos.	prev.	N pos.	prev.	N pos.	prev.
Testing delay (in days)							
≤1	1180	102	8.6%	96	8.1%	6	0.5%
2-4	699	74	10.6%	66	9.4%	8	1.1%
5-7	979	69	7.0%	65	6.6%	4	0.4%
>7	615	172	28.0%	157	25.5%	15	2.4%
Quarter of sampling							
January-March	548	57	10.4%	57	10.4%	0	0.0%
April-June	841	87	10.3%	77	9.2%	10	1.2%
July-September	854	110	12.9%	104	12.2%	6	0.7%
October-December	1230	163	13.3%	146	11.9%	17	1.4%
Holding type							
Farrow to finish	1868	214	11.5%	198	10.6%	16	0.9%
Farrow to weaner	350	44	12.6%	40	11.4%	4	1.1%
Farrow to grower	1255	159	12.7%	146	11.6%	13	1.0%
Holding size							
<100	971	70	7.3%	69	7.1%	1	0.1%
100-399	1419	205	14.4%	190	13.4%	15	1.1%
400-999	656	88	13.4%	75	11.4%	13	2.0%
>999	427	54	12.6%	50	11.7%	4	0.9%
Gilt replacement policy							
>90% gilts home-bred	1539	160	10.4%	153	9.9%	7	0.5%
10-90% gilts home-bred	470	45	9.6%	41	8.7%	4	0.9%
>90% gilts purchased	1464	212	14.5%	190	13.0%	22	1.5%
Boar replacement policy							
no boars on farm	408	49	12.0%	45	11.0%	4	1.0%
>90% home-bred	982	142	14.5%	136	13.8%	6	0.6%
10-90% purchased	253	31	12.3%	27	10.7%	4	1.6%
>90% purchased	1830	195	10.7%	176	9.6%	19	1.0%

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

Breeding pig holding



Production pig holding

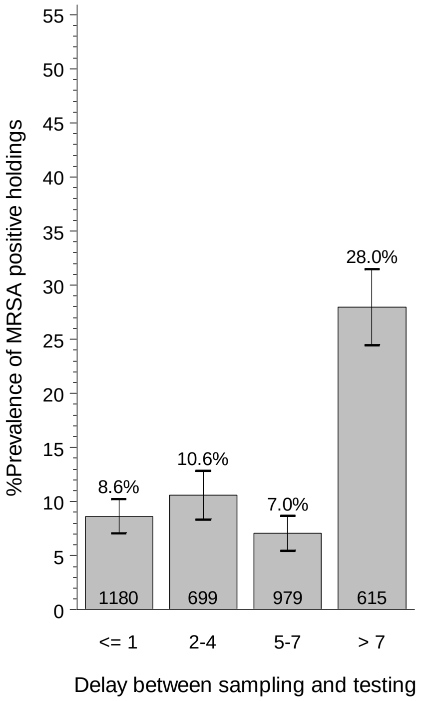
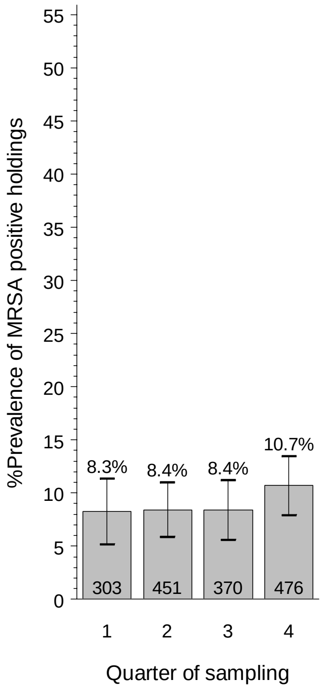


Figure 8: MRSA prevalence with 95% CI by testing delay (days) (number of holdings represented inside each bar), MRSA EU baseline survey in breeding pigs, 2008

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

Breeding pig holding



Production pig holding

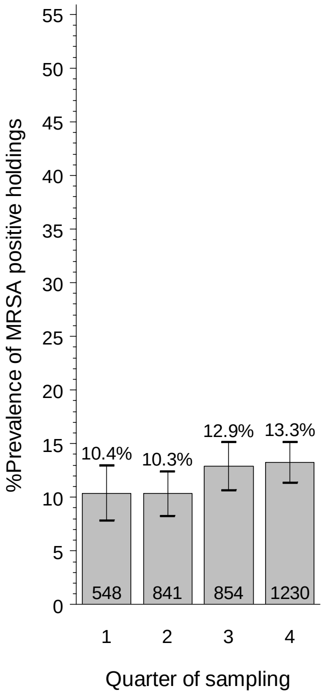
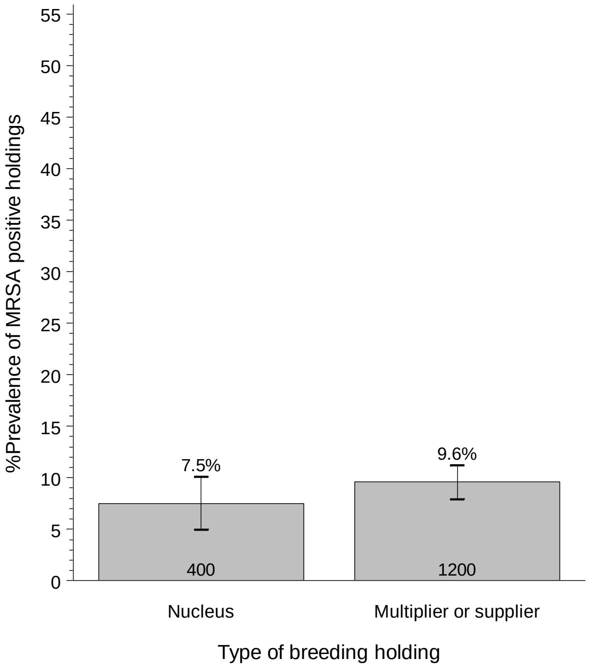


Figure 9: MRSA prevalence with 95% CI by quarter of sampling (number of holdings represented inside each bar), MRSA EU baseline survey in breeding pigs, 2008[(a)]

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

Breeding pig holding



Production pig holding

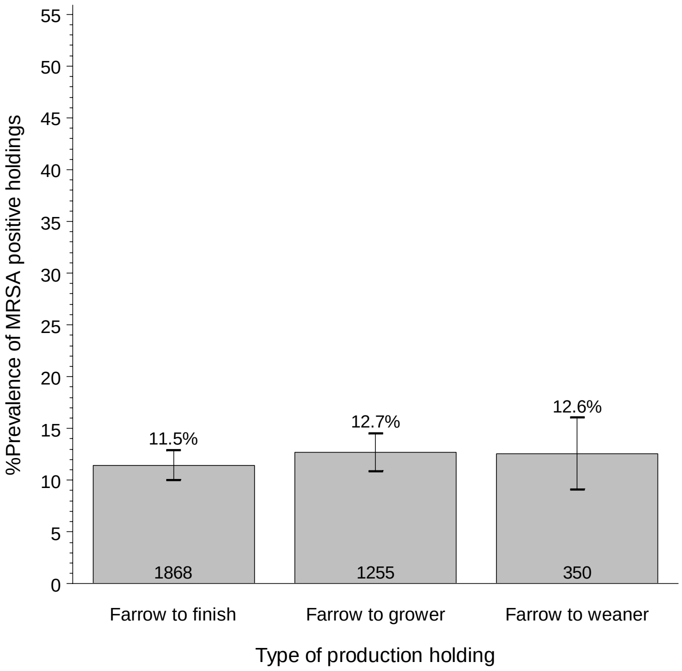
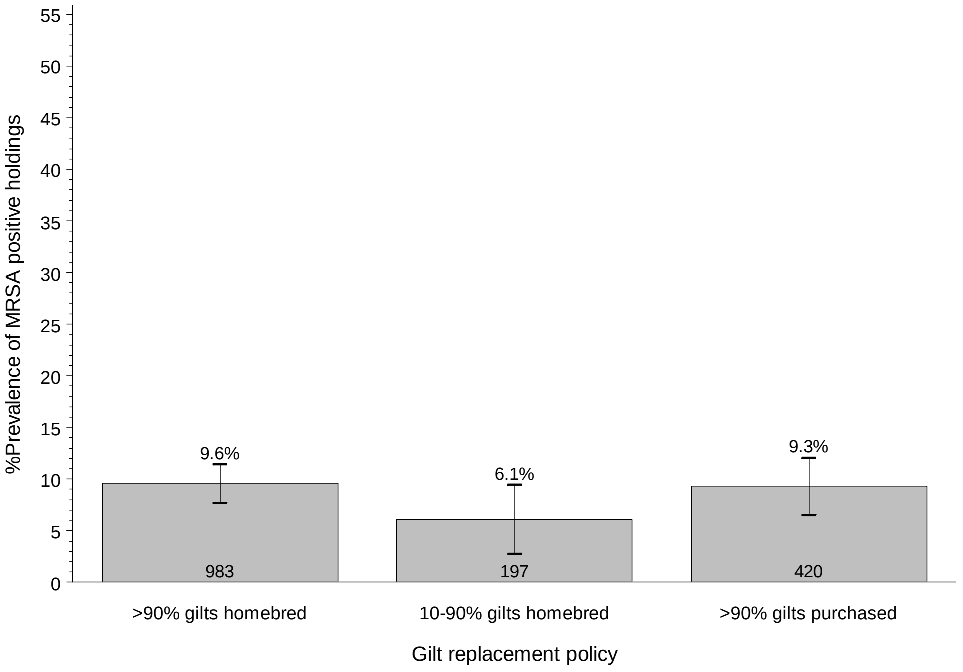


Figure 10: MRSA prevalence with 95% CI by type of holding (number of holdings represented inside each bar), MRSA EU baseline survey in breeding pigs, 2008[(a)]

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

Breeding pig holding



Production pig holding

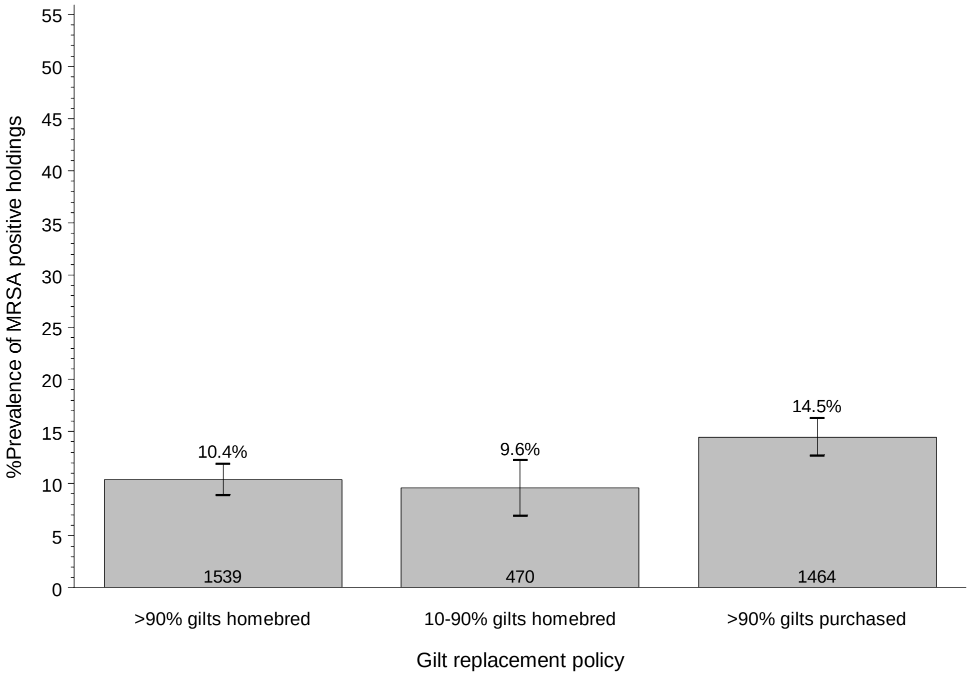
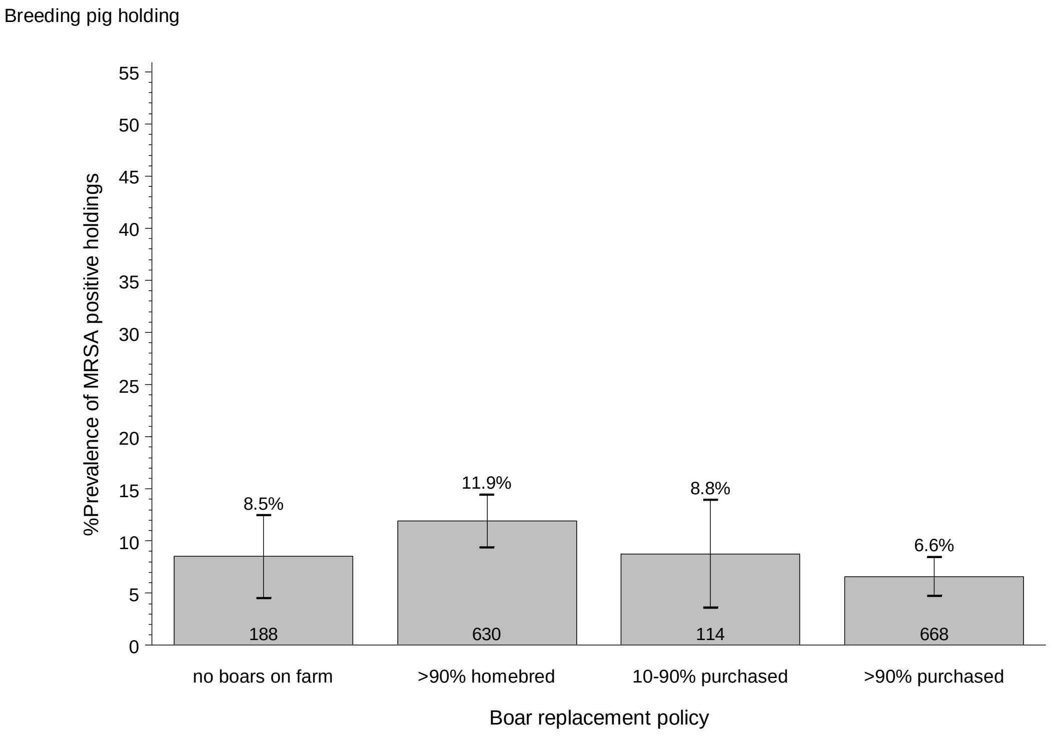


Figure 11: MRSA prevalence with 95% CI by gilt replacement policy (number of holdings represented inside each bar), MRSA EU baseline survey in breeding pigs, 2008[(a)]

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



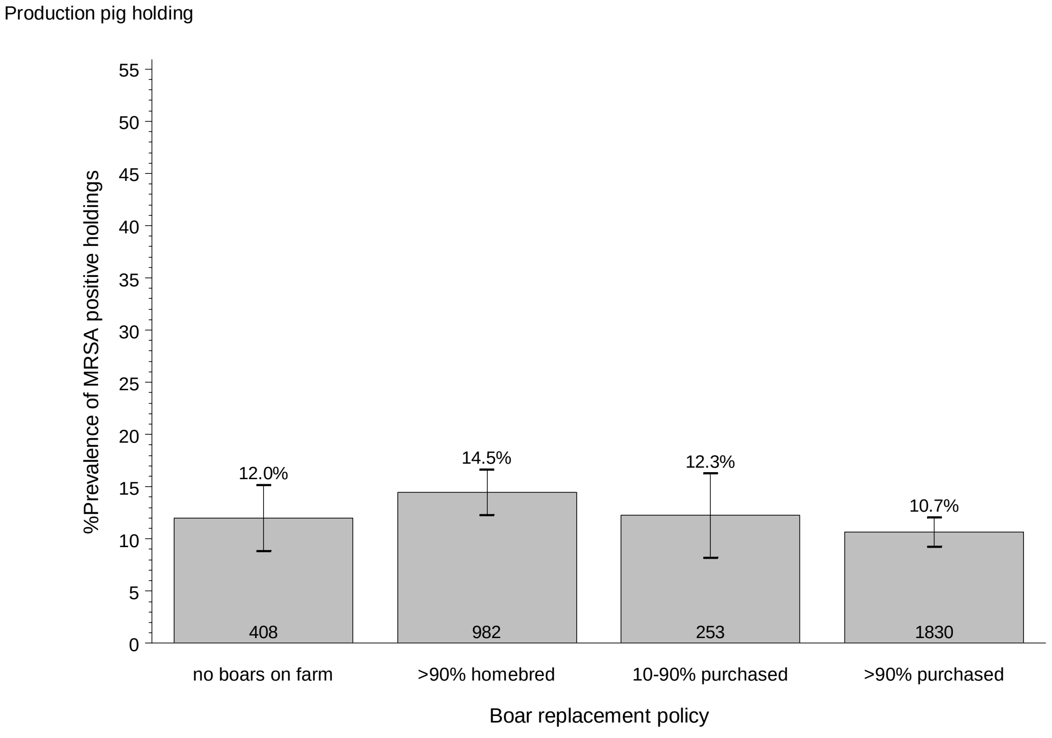


Figure 12: MRSA prevalence with 95% CI by boar replacement policy (number of holdings represented inside each bar), MRSA EU baseline survey in breeding pigs, 2008[(a)]

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

Table 8: Bivariable odds ratios of MRSA contamination by risk factor, from a model with country as random effect; MRSA baseline survey in breeding pigs, 2008[(a)]

Risk factor	Breeding holding			Production holding		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Sampling quarter			0.2377			0.4452
January-March	1	-		1	-	
April-June	0.68	0.42, 1.09		0.75	0.51, 1.11	
July-September	0.62	0.36, 1.07		0.94	0.54, 1.62	
October-December	0.75	0.39, 1.44		0.97	0.54, 1.73	
Type of breeding holding			0.1625			-
Multiplier	1	-		-	-	
Nucleus	0.64	0.35, 1.20		-	-	
Type of production holding			-			0.7804
Farrow-to-finish	-	-		1	-	
Farrow-to-grower	-	-		0.93	0.75, 1.15	
Farrow-to-weaner	-	-		1.02	0.65, 1.59	
Size of the holding			0.0080			0.0042
<100	1	-		1	-	
100-399	1.96	0.95, 4.04		1.86	1.14, 3.04	
400-999	2.74	1.29, 5.85		2.22	1.34, 3.66	
>999	2.54	1.33, 4.87		2.58	1.41, 4.70	
Gilt replacement policy			0.0074			0.1851
>90% gilts home-bred	1	-		1	-	
10-90% gilts home-bred	0.84	0.40, 1.77		1.31	0.93, 1.84	
>90% gilts purchased	1.61	1.02, 2.53		1.29	0.96, 1.75	
Boar replacement policy			0.4127			0.1555
No boar on farm	1	-		1	-	
>90% home-bred	1.18	0.65, 2.14		0.54	0.31, 0.96	
10-90% purchased	0.67	0.33, 1.36		0.58	0.34, 0.99	
>90% purchased	0.91	0.43, 1.91		0.54	0.30, 0.97	
Testing delay (in days)			0.2447			0.0192
≤1	1	-		1	-	
2-4	0.82	0.43, 1.55		1.07	0.52, 2.19	
5-7	1.56	0.51, 4.80		0.77	0.37, 1.59	
>7	1.83	0.77, 4.34		1.26	0.56, 2.84	

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

G. RISK FACTOR ANALYSIS: FULL MODEL USED AS STARTING POINT OF THE BACKWARD STEPWISE PROCEDURE FOR FITTING THE FINAL MODEL[(a)]

Table 9: Full random effect logistic models for factors associated with MRSA contamination in breeding holdings and in production holdings, MRSA EU-baseline survey in breeding pigs, 2008 [(a)]

Variables	Full Random effect logistic models					
	Breeding holdings[(b)]			Production holdings[(b)]		
	OR	95%CI	*P*-value	OR	95%CI	*P*-value
Testing delay (in days)			0.47			0.21
0-1day	1	-		1	-	
2-4 days	0.88	0.38, 2.02		1.08	0.48, 2.4	
5-7 days	1.74	0.48, 6.28		0.81	0.36, 1.85	
>7days	1.91	0.68, 5.38		1.28	0.5, 3.29	
Quarter of sampling			0.58			0.66
January-March	1	-		1	-	
April-June	0.71	0.38, 1.31		0.78	0.49, 1.25	
July-September	0.63	0.31, 1.26		0.92	0.51, 1.64	
October-December	0.73	0.35, 1.51		0.99	0.51, 1.93	
Holding type			0.26			0.86
Multiplier or supplier	1	-		-	-	
Nucleus	0.67	0.34, 1.33		-	-	
Farrow-to-finish	-	-		1	-	
Farrow-to-grower	-	-		0.94	0.74, 1.21	
Farrow-to-weaner	-	-		0.91	0.54, 1.52	
Holding size (in breeding pigs)			0.04			0.04
<100	1	-		1	-	
100-399	1.94	0.82, 4.55		1.82	1.04, 3.20	
400-999	2.83	1.21, 6.59		2.16	1.19, 3.92	
>999	2.75	1.16, 6.53		2.55	1.23, 5.32	
Gilt replacement policy			0.049			0.21
>90% gilts home-bred	1	-		1	-	
10-90% gilts home-bred	0.96	0.38, 2.42		1.37	0.88, 2.13	
>90%gilts purchased	1.82	1.05, 3.15		1.27	0.94, 1.73	
Boar replacement policy			0.29			0.25
No boar on farm	1	-		1	-	
>90% boar home-bred	1.48	0.67, 3.26		0.57	0.31, 1.05	
10-90% boar home-bred	0.83	0.31, 2.22		0.59	0.31, 1.13	
>90%boar purchased	0.88	0.37, 2.13		0.54	0.29, 1.00	

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

(b): Estimates and standard errors were assessed using a logistic mixed model with a country random effect on the intercept (*P*-value < 0.0001).

H. INTRA-COMMUNITY TRADE OF BREEDING PIGS

Analysis of trade data

To analyse the relation between among-MS trade and MRSA prevalence, random intercept (multilevel) logistic models were fitted with country as random intercept, and the import volume of breeding pigs into a country as an independent variable. Other independent variables tested in the random intercept logistic model were the percentage of imported breeding pigs, the number of shipments of breeding pigs into a MS, and the number of trading partners (countries of origin) from which a MS imported breeding pigs. The likelihood ratio test was used to test whether the effect of trade was statistically significant. We also added holding size as an extra covariate to these models. This did not change results importantly. The association between the MRSA holding contamination and the import volume into a country of breeding pigs at risk of being colonised was assessed using a similar logistic random effect model as previously described. For the purpose, a proxy-number of imported breeding pigs at risk of being colonised was estimated per year and per MS, as shown below:

$$\text{Proxy-No}_{\text{imported breeding pigs at risk of colonisation}} = \sum\nolimits_{\text{country of origin}} (\text{No}_{\text{imported breeding pigs}} \times \text{MRSA Prevalence}_{\text{holdings with breeding pigs}})$$

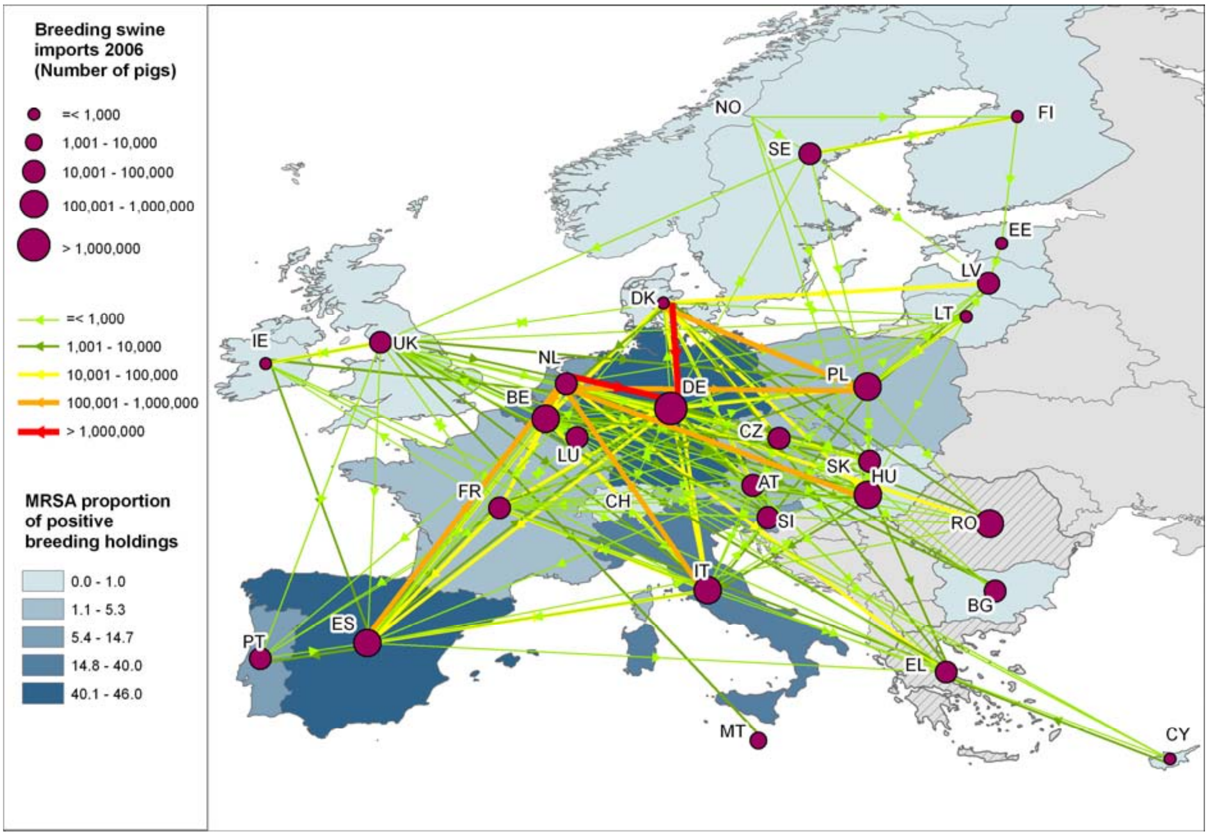


Figure 13: Prevalence of MRSA-positive breeding holdings in 2008 (EFSA, 2009) and within-Community trades of breeding pigs in 2006(a) [14]

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

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[14] Lines connecting Country A and Country B are represented with a specific colour (green to red where red represents larger numbers) and thickness (increasing thickness corresponds to larger numbers) to display the amount of imports among the MSs. Arrows along the route indicate direction of flux. A dot for each country is represented with a specific size based on the total number of breeding pigs imported by each country (larger dots represent larger numbers).

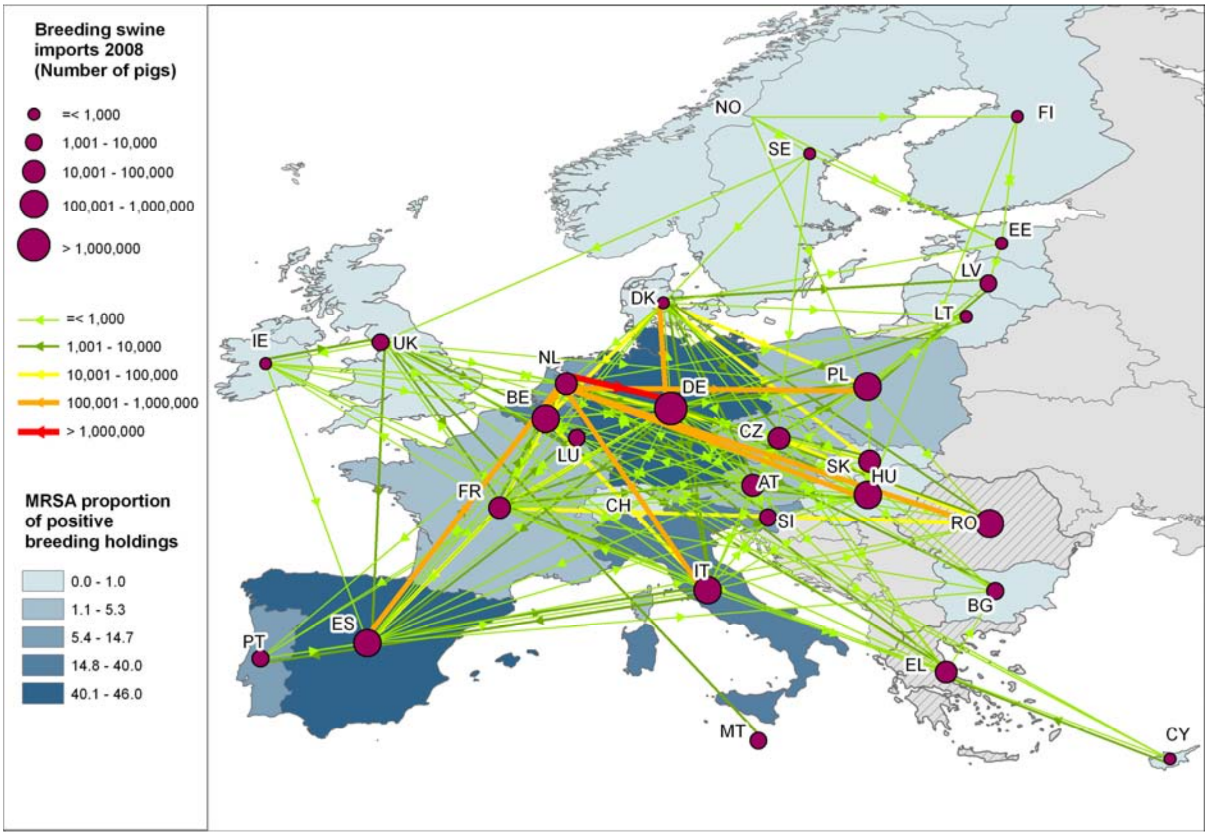

Figure 14: Prevalence of MRSA-positive breeding holdings in 2008 (EFSA, 2009) and within-Community trades of breeding pigs in 2008(a) [15]

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

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[15] Lines connecting Country A and Country B are represented with a specific colour (green to red where red represents larger numbers) and thickness (increasing thickness corresponds to larger numbers) to display the amount of imports among the MSs. Arrows along the route indicate direction of flux. A dot for each country is represented with a specific size based on the total number of breeding pigs imported by each country (larger dots represent larger numbers).

Table 10: Spearman correlation coefficients and corresponding *P*-values testing the correlation between the MRSA prevalence of holdings with breeding pigs in 2008 and data on breeding pigs trade in 2006-2008

Holding Type	Year	Type of trade data[(a)]	Spearman ρ	*P*-value[(b)]
All holdings	**2006-2008**	Number of imported breeding pigs	0.60	0.0018
		Percentage of imported pigs	0.52	0.009
		Number of consignments imported	0.69	0.0002
		Number of trading partners [(c)]	0.55	0.0054
	2006	Number of imported breeding pigs	0.60	0.002
		Percentage of imported pigs	0.45	0.026
		Number of consignments imported	0.67	0.0003
		Number of trading partners	0.51	0.0101
	2007	Number of imported breeding pigs	0.67	0.0004
		Percentage of imported pigs	0.58	0.0032
		Number of consignments imported	0.75	<.0001
		Number of trading partners	0.58	0.0029
	2008	Number of imported breeding pigs	0.61	0.0014
		Percentage of imported pigs	0.54	0.0071
		Number of consignments imported	0.68	0.0003
		Number of trading partners	0.52	0.0096
Breeding Holdings	**2006-2008**	Number of imported breeding pigs	0.71	0.0001
		Percentage of imported pigs	0.48	0.0186
		Number of consignments imported	0.82	<.0001
		Number of trading partners	0.68	0.0002
	2006	Number of imported breeding pigs	0.70	0.002
		Percentage of imported pigs	0.42	0.0407
		Number of consignments imported	0.79	<.0001
		Number of trading partners	0.65	0.0007
	2007	Number of imported breeding pigs	0.73	<.0001
		Percentage of imported pigs	0.53	0.0079
		Number of consignments imported	0.81	<.0001
		Number of trading partners	0.71	0.0001
	2008	Number of imported breeding pigs	0.74	<.0001
		Percentage of imported pigs	0.56	0.0045
		Number of consignments imported	0.78	<.0001
		Number of trading partners	0.63	0.0009
Production Holdings	**2006-2008**	Number of imported breeding pigs	0.54	0.0068
		Percentage of imported pigs	0.46	0.0247
		Number of consignments imported	0.63	0.0009
		Number of trading partners	0.47	0.0204
	2006	Number of imported breeding pigs	0.54	0.0064
		Percentage of imported pigs	0.40	0.0528
		Number of consignments imported	0.67	0.0003
		Number of trading partners	0.44	0.0333
	2007	Number of imported breeding pigs	0.60	0.0018
		Percentage of imported pigs	0.52	0.0096
		Number of consignments imported	0.70	0.0001
		Number of trading partners	0.51	0.0106
	2008	Number of imported breeding pigs	0.54	0.0069
		Percentage of imported pigs	0.46	0.0244
		Number of consignments imported	0.61	0.0017
		Number of trading partners	0.44	0.0320

(a): Imported breeding pigs/lots/partners also include trade from Norway and Switzerland to EU MSs.

(b): *P*-values testing the null hypothesis of no association between MRSA prevalence estimates and breeding pig trade.

(c): Countries of origin.

Table 11: Strength and significance of the association between MRSA holding contamination in 2008 and volume of intra-Community breeding pig imports in 2006-2008

Holdings	Year	Type of trade data[(a)]	% variance explained	*P*-value[(b)]
All holdings	**2006-2008**	Number of imported breeding pigs	25.0%	0.0233
		Percentage of imported pigs	29.3%	0.0104
		Number of consignments imported	28.6%	0.0146
		Number of trading partners[(c)]	22.4%	0.0130
	2006	Number of imported breeding pigs	17.4%	0.0242
		Percentage of imported pigs	22.3%	0.0234
		Number of consignments imported	28.2%	0.0164
		Number of trading partners	21.7%	0.0117
	2007	Number of imported breeding pigs	30.2%	0.0116
		Percentage of imported pigs	36.9%	0.0035
		Number of consignments imported	33.1%	0.0078
		Number of trading partners	26.6%	0.0074
	2008	Number of imported breeding pigs	22.0%	0.0258
		Percentage of imported pigs	16.6%	0.0521
		Number of consignments imported	19.2%	0.0177
		Number of trading partners	21.7%	0.0117
Breeding holdings	**2006-2008**	Number of imported breeding pigs	27.7%	0.0030
		Percentage of imported pigs	23.6%	0.0589
		Number of consignments imported	32.6%	0.0016
		Number of trading partners	29.2%	0.0047
	2006	Number of imported breeding pigs	25.4%	0.0041
		Percentage of imported pigs	14.3%	0.1996
		Number of consignments imported	33.2%	0.0017
		Number of trading partners	28.0%	0.0039
	2007	Number of pigs imported	36.1%	0.0013
		Percentage of imported pigs	35.0%	0.0207
		Number of consignments imported	39.4%	0.0008
		Number of trading partners	34.9%	0.0043
	2008	Number of imported breeding pigs	24.1%	0.0038
		Percentage of imported pigs	19.3%	0.0451
		Number of consignments imported	33.3%	0.0139
		Number of trading partners	28.0%	0.0039
Production holdings	**2006-2008**	Number of imported breeding pigs	23.6%	0.0240
		Percentage of imported pigs	29.6%	0.0152
		Number of consignments imported	22.7%	0.0128
		Number of trading partners	20.3%	0.0166
	2006	Number of imported breeding pigs	21.7%	0.0312
		Percentage of imported pigs	23.1%	0.0329
		Number of consignments imported	26.9%	0.0173
		Number of trading partners	18.9%	0.0185
	2007	Number of imported breeding pigs	29.2%	0.0121
		Percentage of imported pigs	38.3%	0.0047
		Number of consignments imported	31.9%	0.0082
		Number of trading partners	24.9%	0.0084
	2008	Number of imported breeding pigs	17.7%	0.0300
		Percentage of imported pigs	15.9%	0.0738
		Number of consignments imported	18.2%	0.0185
		Number of trading partners	18.9%	0.0185

(a): Imported breeding pigs/lots/partners also include trade from Norway and Switzerland to EU MSs.

(b): *P*-values testing the null hypothesis of no association between MRSA prevalence estimates and breeding pig trade.

(c): Countries of origin.

I. SPATIAL DISTRIBUTION OF MRSA SPA-TYPES IN THE EU

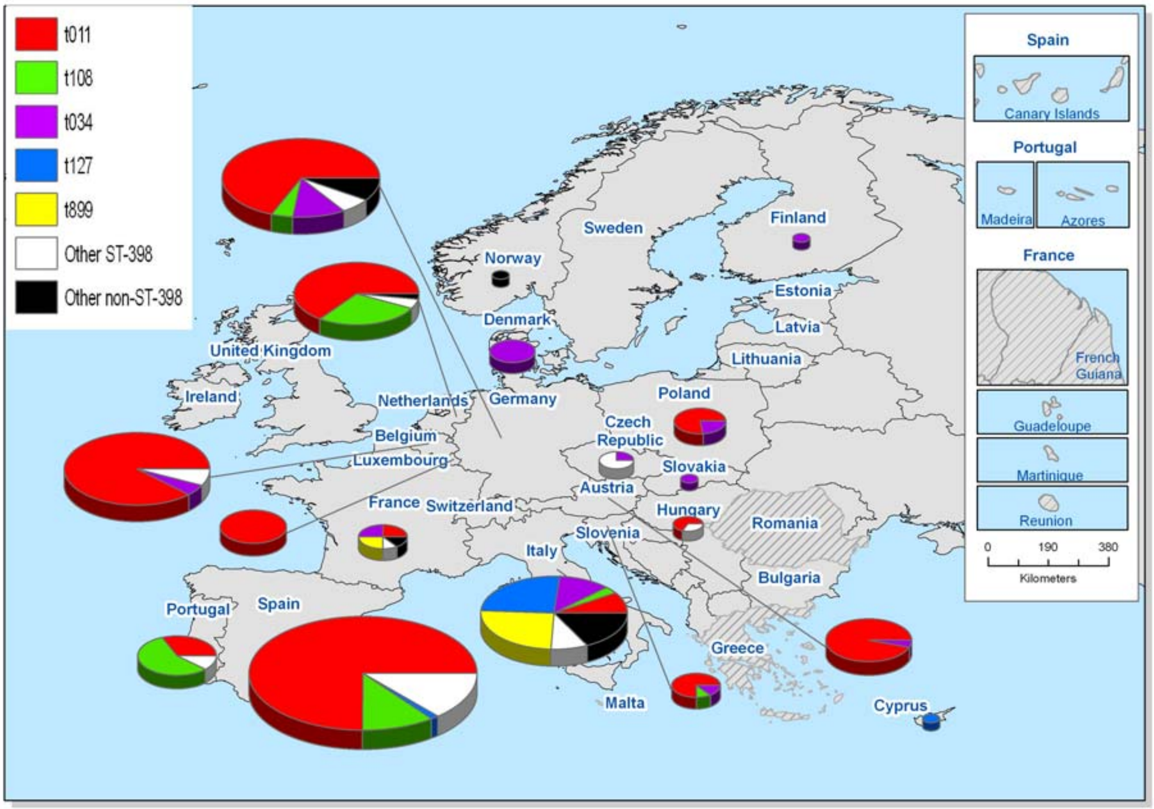

Figure 15: The distribution of *spa*-types of MRSA[(a)] in environmental dust samples collected from breeding and production holdings in the EU[(b)], Norway and Switzerland, 2008

(a): Pie chart sizes illustrate the number of MRSA isolates isolated in each country.

(b): Greece, Malta and Romania did not carry out the baseline survey: they are visualised on the map with dashed fill symbology.

GLOSSARY

Breeding pig	A breeding pig means a pig (sow or boar) of at least six months of age kept for breeding purposes.
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 *S. aureus* 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 10-12 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)[16]	Multi-Locus Sequence Typing is a molecular typing method that allows the checking for nucleotide differences between isolates of microbes. In the case of *Staphylococcus aureus*, including MRSA strains, it is used to identify the seven housekeeping genes present in all *S. aureus* 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.
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 apparent and true prevalence is the function of the sensitivity and the specificity of the diagnostic test used.
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.

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[16] 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).

RIDOM StaphType Database (www.spaSpaserver.ridom.de)	Single locus DNA-sequencing of the repeat region of the *Staphylococcus* protein A gene (*spa*) can be used for reliable, accurate and discriminatory typing of MRSA. Repeats are assigned a numerical code and the *spa*-type is deduced from the order of specific repeats. However, *spa*-typing was hampered in the past by the lack of a consensus on assignments of new *spa*-repeats and *spa*-types. 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 *spa*-repeat sequences and the *spa*-types can be downloaded. Chromatograms of new *spa*-repeats and/or -types can be submitted online for inclusion in the reference database.
Sensitivity	Ability of a test to detect correctly epidemiological units (e.g. animals, holdings) with the disease or infection of interest.
Sow	A female pig that has had a litter.
Spa-typing[17]	A molecular typing method used for the subtyping of *S. aureus* including MRSA and subsequently for identifying MRSA lineage. This technique involves PCR amplification and sequencing of the variable region of the protein A (*spa*) gene, which encodes the staphylococcal protein A located on the chromosome of all *S. aureus*. The variable region of the *spa* gene consists of specific repeated DNA sequences (called "repeats"). The *spa*-typing method assigns alpha-numerical codes to different repeats, and based on their order and composition, *spa* repeat sequences are automatically assigned a *spa*-type by submission to the RIDOM StaphType Database (www.spaSpaserver.ridom.de). The most likely sequence type of new *spa*-types can often be inferred by comparing to well-defined *spa*-types with close *spa* repeat homology for which the ST has already been determined by MLST typing. MRSA with the same *spa*-type will, in most cases, belong to the same sequence type/clonal complex.
Specificity	Ability of a test to correctly detect epidemiological units (e.g. animals, holdings) 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 which has been removed from the sow.

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[17] 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

CA-MRSA	Community-acquired MRSA
CC	Clonal Complexes
CI	Confidence Interval
EU-RL-AR	Community Reference Laboratory for Antimicrobial Resistance
EC	European Commission
EFSA	European Food Safety Authority
EFTA/EEA	European Free Trade Association/European Economic Area
EU	European Union
HA-MRSA	Health-care-associated MRSA
MLST	Multi-Locus Sequence Typing
LA-MRSA	Livestock-associated MRSA
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MS	Member State
MSSA	Methicillin-Sensitive *Staphylococcus aureus*
NRL	National Reference Laboratory
OIE	Office International des Epizooties
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PVL	Panton-Valentine leukocidin
QA	Quality Assurance
SCCmec	Staphylococcal cassette chromosome mec
spa-typing	*Staphylococcus* protein A typing
ST	Sequence type
ST398	MRSA lineage multi-locus sequence type 398
TRACES	TRAde Control and Expert System
TSB	Tryptone Soy Broth
VIF	Variance Inflation Factor