Epidemiological evaluation of the residue surveillance program in Danish pigs

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PREFACE

In 1972, the Danish Veterinary and Food Administration started the official residue surveillance program in pigs. Additionally, from 2001 onwards, Danish abattoirs have randomly sampled sows and slaughter pigs for antibacterial residues. Each year, more than 20,000 samples are analysed for presence of antibacterial residues in Danish pigs, which corresponds to 0.1% of the size of the population of slaughter pigs and more than 1% of the size of the population of sows slaughtered, exceeding the 0.03% sampling level required by European Union authorities. Data collected over the last ten years indicate that antibacterial residues in Danish pigs are found at a very low prevalence.

Given the very low prevalence of antibacterial residues in Danish pork, in combination with the current high focus on food safety in Denmark along with export requirements, the question arose whether the current Danish residue surveillance program could be revised to be more cost-effective. This implies a more effective allocation of resources, reducing the overall costs, while assuring high standards for the protection of human health and the exports.

To address this question, four initiatives were taken. First, a risk assessment was conducted to evaluate the human health risk of residues in Danish pork. Secondly, an epidemiological evaluation of the current surveillance system and impact of using alternative antibacterial residue screening tests was made. Thirdly, an epidemiological evaluation of the impact of introducing a risk-based approach to slaughter pig antibacterial residue surveillance was conducted. And finally, studies were identified which are required to perform a throughout evaluation of the epidemiological and economic consequences of introducing alternative screening tests and sampling schemes in the antibacterial residue surveillance program in Denmark.

SUMMARY

Use of veterinary medicinal products in food-producing animals might result in presence of residues in food products from these animals. Hence, humans might be exposed to residues and this might result in harmful consequences.

A qualitative risk assessment was conducted, according to international guidelines, to evaluate the human health risk of residues in Danish pork. The hazard identification step identified the residues that could potentially be found in Danish pork. The release assessment evaluated the probability of release of antibacterial residues in Danish pork, based on antibacterial consumption data covering 2008. The exposure assessment estimated the probability of human exposure, based on antibacterial residue surveillance data in Danish pigs (covering 2005-2009). The consequence assessment evaluated the potential public health consequences and likelihood of its occurrence, based on a literature search. Finally, a risk estimate combined release, exposure and consequence assessment. The human health risk associated with antibacterial residues in Danish pork was considered low to negligible in sows and negligible in slaughter pigs. Still, reasons other than food safety might also apply and require that surveillance activities are in place. This includes public perception of animal health and welfare and food safety, industry reputation and, most of all, compliance with European legislation and export requirements.

A Bayesian epidemiological model was developed to evaluate the Danish antibacterial residue surveillance system accuracy, i.e., the ability to correctly identify pigs presenting residues above the maximum residue limits (MRLs). The consequences of introducing alternative screening tests and sampling schemes were also evaluated. Overall, true antibacterial residue prevalence in Danish pork is very low. Even though sows present a 10-25 times higher risk of having residues above the MRLs compared to slaughter pigs, the human health risk associated with antibacterial residues in Danish sows was considered low and even negligible in slaughter pigs. To further reduce the already very low prevalence of antibacterial residues in Danish sows, increased focus on good management practices regarding antibacterial use should be advocated for. This will help preventing treated sows being delivered to slaughter before the end of the withdrawal period. Additionally, education of farmers and farm workers could also be promoted to increase awareness regarding the impact of potential detection of antibacterial residues above the MRLs on industry reputation and above all, exports.

Results suggest that the current screening test used in Denmark (the 4-plate method) presents high sensitivity and very high specificity for the most relevant antibacterial classes used in Danish pigs. However, if export requirements demand for limits below the MRLs established in the EU, alternative screening tests should be investigated.

The current sample size used in the own-check antibacterial residue surveillance in slaughter pigs (~20,000 samples) allows for very high sampling sensitivity, i.e., very high probability of detecting at least one slaughter pig presenting residues above the MRLs. However, given the

very low to negligible prevalence found in slaughter pigs, sampling sensitivity is significantly reduced, if sample size is reduced to less than 15,000 samples. If high-risk slaughter pigs could be identified, a risk-based approach to slaughter pig surveillance would allow reducing sample size, while increasing sampling sensitivity.

Model results also illustrated that screening tests significantly impact the Danish antibacterial residue surveillance sensitivity. To detect pigs presenting residues above the MRLs and avoid too many false positive pigs, a screening test should ideally present very high sensitivity and very high specificity. Alternatively, a screening test with very high sensitivity and a post-screening test with high sensitivity and specificity should be used in combination.

To both comply with export requirements and increase the cost-effectiveness of the current antibacterial residue surveillance program in Danish pigs, further specific studies are required. These include:

1. An evaluation of alternative screening and post-screening test characteristics (sensitivity and specificity, time, costs and complexity of testing procedure),
2. An investigation of a risk-based surveillance approach including information on antibacterial use and post-mortem meat inspection.

SAMMENDRAG

Anvendelse af veterinære lægemidler til produktionsdyr kan medføre tilstedeværelse af reststoffer i kød og mælk. Mennesker kan dermed blive eksponerede for reststoffer i forbindelse med konsumering af mælk og kød, og dette kan have negative konsekvenser.

Den humane risiko forbundet med reststoffer i dansk svinekød er estimeret ved hjælp af en kvalitativ risikovurdering, udført i overensstemmelse med internationale guidelines. Først er det identificeret, hvilken type af reststoffer, der potentielt kan findes i dansk svinekød (hazard identifikation). Dernæst er sandsynligheden for at møde disse stoffer i de levende svin estimeret på baggrund af data vedrørende forbrug af antibiotika i 2008 (release vurdering). Sandsynligheden for human eksponering er vurderet ud fra data fra reststofovervågningen i perioden 2005-2009 (eksponeringsvurdering). Konsekvenserne af en eksponering er vurderet ved hjælp af et litteraturstudium og der er set på sandsynlighed og alvor af symptomer (konsekvensvurdering). Endeligt blev informationerne om release, eksponering og konsekvenser kombineret til et risikoestimat.

Den humane sundhedsrisiko forbundet med reststoffer i dansk svinekød vurderes som lav for svin og ubetydelig for slagtesvin. Der er dog andre årsager til, at der er behov for overvågning for reststoffer. Dette inkluderer en offentlig opfattelse af, hvad dyresundhed og dyrevelfærd er, hvad fødevaresikkerhed er, erhvervets ry og sidst men ikke mindst dokumentation af overholdelse af EU lovgivning og eksport krav.

En Bayesiansk epidemiologisk model blev udviklet med henblik på at evaluere præcisionen af det danske program for reststofovervågning. Dette vil sige evnen til korrekt at udpege svin, som har reststoffer i kroppen over den maksimale reststofgrænse (MRL-værdi). Betydningen af at introducere alternative laboratoriemetoder som screeningstests eller alternative udpegningsplaner blev også evalueret. Overordnet set er den sande forekomst af reststoffer i dansk svinekød meget lav. Selv om søer repræsenterer en 10-25 gange større risiko for at have reststoffer over MRL-værdien sammenlignet med slagtesvin, så er den humane sundhedsrisiko forbundet med reststoffer i danske søer lav. Og i slagtesvin er den endda ubetydelig. For at mindske den lave forekomst af reststoffer i danske søer kan man rette yderligere fokus på håndtering og brug af antibiotika. Hermed kan man forebygge, at behandlede søer leveres til slagtning, før end tilbageholdelsestiden er udløbet. Kurser og videreuddannelse af landmænd og deres personale kan også anbefales for at øge opmærksomhed på betydning af fund af reststoffer over MRL-værdien for erhvervets ry og herunder særligt eksporten.

Resultaterne indikerer, at den nuværende screeningstest brugt i Danmark (4-plade metoden) har en høj sensitivitet og en meget høj specificitet for hovedparten af de relevante antibiotikagrupper brugt til danske svin. Men hvis der er behov for lavere grænser end MRL-værdierne (EU's grænseværdier), så kan man med fordel evaluere alternative screenings tests.

Den nuværende stikprøvestørrelse der bruges i virksomhedernes egenkontrolprogram for slagtesvin (~20.000 prøver) resulterer i en meget høj sensitivitet, her forstået som en meget høj sandsynlighed for at detektere i hvert fald ét slagtesvin over MRL-værdien. Men fordi forekomsten i slagtesvin er meget lav til ubetydelig, så reduceres sensitiviteten af overvågningen, hvis stikprøven mindskes til under 15.000 samples. Hvis høj-risiko slagtesvin kan identificeres på baggrund af en risiko-baseret tilgang, kan man reducere stikprøven samtidig med, at sensitiviteten af programmet øges.

Modelresultater viser også, at screeningstesten har en betydelig indvirkning på sensitiviteten af det danske overvågningsprogram. For at finde svin med reststoffer over MRL-værdien og samtidig undgå for mange falsk-positive svin, skal en screeningstest ideelt set både have meget høj sensitivitet og meget høj specificitet. Alternativt kan man i kombination benytte en screeningstest med en meget høj sensitivitet og en post-screeningstest med en høj sensitivitet og specificitet.

Der er behov for yderligere undersøgelse for at dokumentere overholdelse af krav til eksport og øge cost-effektiviteten af det nuværende program for reststofovervåning af svinekød i danske svin. Dette inkluderer:

1. En evaluering af alternative screenings- og post-screeningstest karakteristika (sensitivitet, specificitet, tid til undersøgelse, udgifter og grad af kompleksitet i forbindelse med analyse),

2. En belysning af hvorledes man kan gøre udpegning af svin til undersøgelse mere risiko-baseret ved f.eks. at inkludere information fra besætningen om brug af antibiotika og post-mortem kødkontrol.

1. INTRODUCTION

1.1. Residues of veterinary medicinal products – an overview

Use of veterinary medicinal products in food-producing animals might result in presence of residues of pharmacological active substances or their metabolites in food products from these animals. Additionally, residues might originate from feed, equipment or the environment. Hereby, humans might be exposed to residues via animal products and this might have harmful human health consequences.

Up to the 1960s, addition of antibacterials[1] to foods was used as a common practice to increase shelf life. Tetracyclines were added to fresh poultry and fish, under the premise that antibacterial residues would not be detected after cooking. Because of repeated findings after cooking, this practice was condemned. Addition of antibacterials to milk to reduce bacterial counts and improve the product quality was also accepted in the past (Katz and Brady, 2000).

Antibacterial residue testing was initially concerned with fermentation problems in dairy production. However, as a result of increased use of veterinary medicinal products in food-producing animals, to further assure consumers safety, countries implemented residue surveillance programs. Originally, a zero-tolerance principle enforced that food should not contain any residues of veterinary medicinal products. However, the development and improvement of analytical methods forced to establish new threshold residue limits.

In 1985, the Codex Committee on Residues of Veterinary Drugs in Foods was established to develop international standards for food safety. However, food safety standards vary across countries reflecting different risk perceptions and attitudes towards risk. At the European Union (EU) level, the European Medicines Agency Committee for Veterinary Medicinal Products (EMA) is responsible for the establishment of maximum residue limits (MRLs), while in the United States and Japan, for example, different entities assume that role (the Food and Drug Administration in the United States and the Pharmaceutical and the Food Safety Bureau of Ministry of Welfare in Japan). Moreover, regulatory and administrative procedures might also vary between importing and exporting countries, posing serious challenges to trade (Wilson et al., 2003).

The EU requires that before the authorisation of a veterinary medicinal product intended for food-producing animals, an extensive evaluation is conducted. This includes pharmacological, toxicological and residue depletion studies and finally, evaluation of analytical methodologies for the identification and quantification of the residues. Based on these studies and on a "standard food basket"[2], an acceptable daily intake (ADI) is determined, representing the level of daily intake during an entire lifetime that poses no risk

[1]Antibiotics (e.g. tetracyclines, macrolides, beta-lactams), sulfonamides and quinolones will be designated as antibacterials throughout this report.

[2]The "standard food basket" assumes that a 60kg adult consumes, on a daily basis, 300g of muscle, 100g of liver, 50g of fat or fat and skin, 50g of kidney, 1500g milk, 100g of eggs and 20g honey.

to consumers. Finally, MRLs are calculated to assure that the daily intake of food with residues will result in a total consumption at or below the ADI (Freischem, 2000). Withdrawal periods for veterinary medicinal products are hence established to assure that concentrations of residues in foods derived from treated animals are not above the MRLs[3].

Allowed substances might have a definitive MRL, a provisional MRL (when further information is required to finalise the evaluation of a substance) or no MRL (when residues at the predicted levels do not constitute a hazard to human health). Substances that might represent a hazard to consumer health at whatever limit must not be used in veterinary medicinal products (prohibited substances) (Regulation (EC) No 470/2009[4]; Commission Regulation (EU) No 37/2010)[5].

To further assure a high level of consumer protection at the EU level, specific legislation regarding surveillance of residues and contaminants in food of animal origin establishes the group of substances to be tested, including the sampling criteria (Council Directive 96/23/EC[6]). Under this legislation, member states are required to have in place national residue surveillance plans, assuring the implementation of specific actions to detect and minimise the recurrence of residues in food of animal origin. Moreover, EU requires that countries have in place a risk-based surveillance program, aiming at increasing the probability of detecting animals presenting residues above the established limits.

1.2. THE DANISH CONTEXT

In 2007, the Danish pig industry comprised approximately 7,000 farmers who produced nearly 26 million pigs. More than 85% of the production was exported to several EU countries including Germany and the United Kingdom, but also to Japan, Russia and the USA (Danske Slagterier, 2008). Danish efforts to produce safe pork include extensive surveillance programs aiming at controlling biological and chemical risks, strict implementation of biosecurity requirements at the pre-harvest level and high hygiene standards at the post-harvest level (Wegener, 2010). Data collected over the last ten years indicate that residues in Danish pork are found at a very low prevalence (Danish Agriculture and Food Council, 2007). Each year, more than 20,000 samples are analysed for presence of residues in Danish pork. The question arose whether the current antibacterial residue surveillance program could be optimized.

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[3]The withdrawal period is determined based on results from residue depletion studies using the formulation indicated by the manufacturer.

[4]Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council. Official Journal L 152, pp. 11-22.

[5] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal L 15, pp. 1-72.

[6]Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products. Official Journal L 125, pp. 10-32.

1.3. AIMS OF THE STUDY

This study aimed at evaluating the human health risk posed by residues in Danish pork. Moreover it was our intention to evaluate the antibacterial residue surveillance program in Danish pigs and the epidemiological consequences of introducing alternative tests and sampling schemes. The use of a risk-based approach to the Danish residue surveillance program was also evaluated.

2. MATERIAL AND METHODS

2.1. THE RESIDUE SURVEILLANCE PROGRAM IN DANISH PIGS

2.1.1. OFFICIAL RESIDUE SURVEILLANCE

In 1972, the Danish Veterinary and Food Administration (DVFA) started the official residue surveillance program in pork[7,8]. At that time, no MRLs were established and hence, all pigs that tested positive for antibacterial residues were immediately condemned.

According to EU regulations, a minimum of 0.05% of the population slaughtered (sows and slaughter pigs) in the previous year should be checked for presence of residues: 0.02% should be checked for substances having an anabolic effect, as well as prohibited substances and 0.03% should be checked for residues of veterinary drugs and contaminants. Regarding antibacterial residues, each year, DVFA samples 0.015% of the number of slaughter pigs slaughtered in the previous year.

According to the annual plan, samples must be collected at the farms (including feed, drinking water and blood, urine or faeces from live animals) and at the abattoirs (muscle, blood or organs). Suspect sampling is conducted in case of suspicion or as consequence of positive findings. All samples are tested at the National Reference Laboratory in Ringsted. Each year, the sampling plan should be revised and changed in view of relevant findings.

Following the detection of residues at a level exceeding the MRLs, the veterinary officer from the regional DVFA service visits the herd. Additionally, the regional veterinary services might issue administrative fines, injunction to revise the farmer’s own check program to assure that the limits can be met in the future and, if necessary, a ban of deliverance for slaughter is put in place. Fines for delivering pigs to slaughter presenting antibacterial residues above the MRLs range from 6,000Dkr to 12,000Dkr, for an average and large herd, respectively.

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[7]Bekendtgørelse om indført forbud mod at levere svin til slagtesteder, såfremt dyrene indeholder antibiotika eller kemoterapeutika. BEK nr. 395 af 25/08/1972.

[8]Bekendtgørelse om dyreejeres anvendelse af lægemidler til dyr og fødevarevirksomheders egenkontrol med restkoncentrationer. BEK nr. 481 af 29/05/2007.

2.1.2. Own check residue surveillance

From 2001 onwards, Danish abattoirs randomly sample sows and slaughter pigs for antibacterial residues, in accordance with the Danish industry code[9]. Each year, the number of slaughter pigs to be examined at Danish abattoirs must be equal to 0.085% of the number of slaughter pigs and 1% of the sows slaughtered in the previous year. At small abattoirs (<1,000 pigs slaughtered/year) one slaughter pig must be examined annually. The antibacterial residue surveillance program in Danish pigs is briefly described in Figure 1. From a randomly selected carcass, one kidney is collected and sent for screening at an accredited laboratory. The carcass, together with the second kidney and 100g of diaphragm or shank (which may not contain any skin, fat or tendons) are detained, while the gut-set and the plucks are condemned. The final disposition of the carcass depends on the laboratory test result. If no residues are detected at screening, the carcass is released and no further action is taken. In case of a positive finding at screening, the second kidney and 100g of diaphragm or shank are sent for confirmation and quantification at the National Reference Laboratory. If the residue level is below or at the MRLs, the carcass can be released. However, because confirmation results can take up to 4-5 weeks, carcasses that test positive at screening are immediately condemned. Additionally, the farmer is informed about the consequences of finding residues above the MRLs, aiming at increasing the farmer's awareness regarding antibacterial use. If the residue level exceeds the MRLs, the entire carcass is condemned. Additionally, a veterinarian from the Danish Agriculture & Food Council visits the herd. At the visit, all conditions concerning use of antibacterials are examined, especially critical points including routines such as storage, usage and recording. If any failures or defects are detected, the veterinarian proposes changes for improvement. The result of the visit is published in a report to the farmer and a copy is sent to the abattoir and the DVFA. The cost of the visit is paid by the farmer (on average 3,000-4,000Dkr).

Suspect sampling might also apply in the following cases:

1) Pigs that have accidentally been delivered before expiry of the withdrawal time;
2) Pigs from farms at risk of antibacterial residues (e.g., farms that have delivered residue positive pigs during the past 2 years).

[9]Branchekode for kontrol med restkoncentrationer i svine-, okse-, fåre- og gedekød. 2009. DwNr. 117560. pp. 1-23.

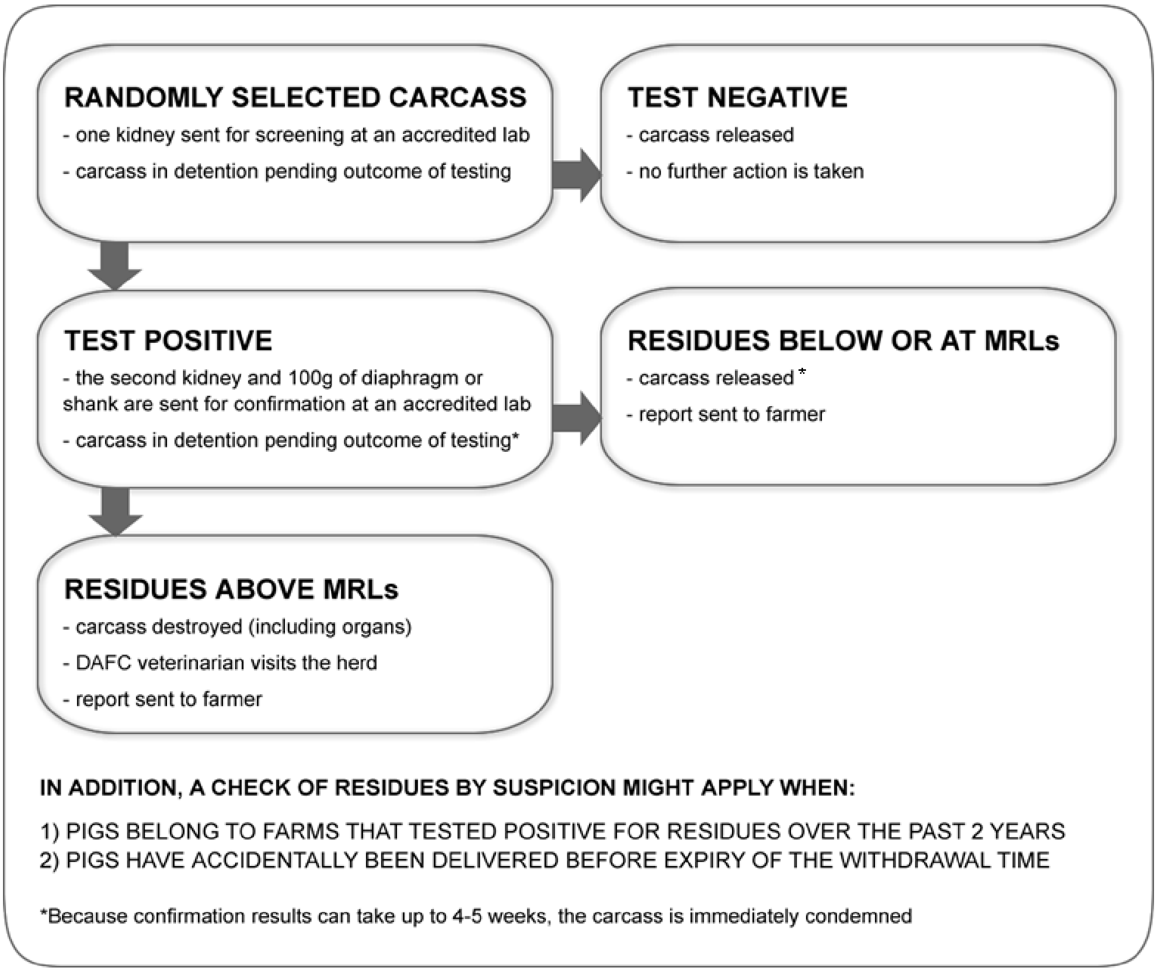

Fig. 1. Overview of the antibacterial residue surveillance program in Danish pig abattoirs.

2.2. SCREENING AND CONFIRMATORY METHODS

Antibacterial residue testing includes both screening and confirmatory methods. Screening methods are used to detect the presence of a substance or classes of substances while confirmatory methods allow identifying the specific substance and, if necessary, quantify it at the level of interest[10].

The total number of samples collected in the Danish official and own check antibacterial residue surveillance program, positive findings at screening and confirmation, from 2005-2009, are presented in Table 1 and Table 2, respectively.

[10]Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal L 251, pp. 8-36.

Table 1

Official antibacterial residue surveillance results in sows and slaughter pigs, 2005-2009, Denmark

Age group	Year	Total number of samples	Number of samples positive at screening	Substances identified (Number)	
				≤ MRLs	> MRLs
Sows	2005	2,062	6[a]	Benzylpenicillin (2) -	Benzylpenicillin (3)
	2006	2,011	5[a]		Benzylpenicillin (3) Lincomycin (1)
	2007	2,014	5	Benzylpenicillin (1)	Benzylpenicillin (3) Amoxicillin (1)
	2008	2,031	11	Benzylpenicillin (3) Tulathromycin (1)	Benzylpenicillin (7)
	2009	2,067	13	Benzylpenicillin (5)	Benzylpenicillin (6) Oxytetracycline (2)
Slaughter pigs	2005	1,002	1[a]	-	-
	2006	1,009	1[a]	-	-
	2007	804	0	-	-
	2008	809	0	-	-
	2009	812	0	-	-

[a]One sample could not be further tested.

Table 2

Own check antibacterial residue surveillance results in sows and slaughter pigs, 2005-2009, Denmark

Age group	Year	Total number of samples	Number of samples positive at screening	Substances identified (N)	
				≤ MRLs	> MRLs
Sows	2005	4,278	10	Benzylpenicillin (5)	Benzylpenicillin (4) Amoxicillin (1)
	2006	3,995	6	Benzylpenicillin (2) Doxycycline (1)	Benzylpenicillin (3)
	2007	5,148	6	Benzylpenicillin (2) Tulathromycin (1)	Benzylpenicillin (3)
	2008	5,422	15[a]	Benzylpenicillin (5)	Amoxicillin (2) Benzylpenicillin (6) Oxytetracycline (1)
	2009	4,445	9	Benzylpenicillin (5)	Benzylpenicillin (4)
Slaughter pigs	2005	18,910	4	The substances could not be identified	
	2006	17,956	2	Amoxicillin (1)	Benzylpenicillin (1)
	2007	17,612	2	Tilmicosin (1)	Benzylpenicillin (1)
	2008	22,806	3	Benzylpenicillin (1)	Doxycycline (1) Benzylpenicillin (1)
	2009	21,686	3[a]	Doxycycline (1)	Doxycycline (1)

[a]One sample could not be further tested.

2.2.1. Screening – the 4-plate method

The 4-plate method was originally developed in 1980 and has been further modified. In Denmark, the 4-plate method developed by the Nordic Committee on Food Analysis is used for screening residues of antibacterial substances. Kidney tissue is submitted directly to four different agar plates containing different sensitive test bacteria in the substrate (*Bacilus subtilis* and *Kocuria rhizophila*, formerly *Micrococcus luteus*), at different pH levels. A test result is considered positive if at least one of the four plates presents an inhibition zone >2mm, after 20-24 hours of incubation. In case of a positive test result, the sample should be tested again on a separate set of plates with tissue form the same kidney. Negative samples should also be retested[11]. Figure 2 presents a schematic representation of the 4-plate method.

[11]Nordic Committee on Food Analysis, No 121. 2004. Antibacterial substances. Microbiological examination of residues of antibiotics and chemotherapeutics in kidney and muscle from carcasses (4-plate method).

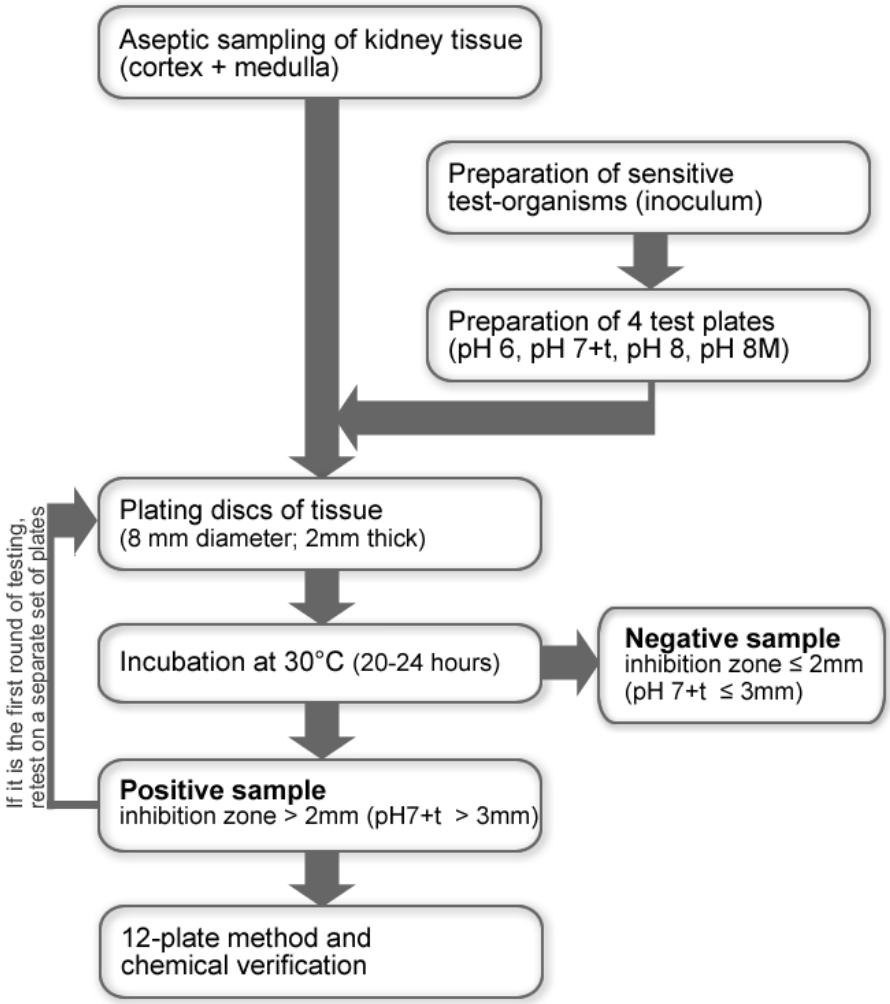

Fig. 2. Schematic representation of the 4-plate method (adapted from Birgitte Herbert Nielsen).

2.2.2. Confirmation

In Denmark, in case of a positive screening test result, the 12-plate method is further used for identification and quantification of the antibacterial agents. Chemical analysis is additionally conducted using high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), photo diode array detection (PDA), gas chromatography-mass spectrometry (GC-MS), fluorescence and ultra-violet (UV) (Gitte Geertsen, personal communication).

2.3. DATA AND METHODS

2.3.1. RISK ASSESSMENT

A qualitative risk assessment was conducted to evaluate the likelihood and the human health consequences of residues in Danish pork, according to international guidelines (Vose et al., 2001):

1) Hazard identification – identification of the residues that could potentially be found in Danish pork were assessed based on Danish residue surveillance data from 2005-2009;

2) Release assessment – the probability of release of antibacterial residues in Danish pork was evaluated based on antibacterial consumption data available from the official register Vetstat (Stege et al., 2003). Animal daily doses (ADDs) in sows and slaughter pigs in 2008 were used to estimate the number of antibacterial doses used per sow and slaughter pig, respectively. The ADD is an assumed average daily dose per animal, defined as the daily maintenance dose for a drug used for its main indication, in a specified species and age group. The ADD is defined for a “standard animal”, i.e., an animal with an estimated average weight within a specified age group: sows/piglets - 200kg and slaughter pigs - 50kg (National Food Institute and Statens Serum Institut, 2009). To obtain an adjusted estimate of the number of doses used, ADDs were further divided by the number of sows (mated sows + sows mated first time, N = 1,020,000) and number of slaughter pigs produced (total pig production minus exported sows and exported weaners, N = 21,900,000) in 2008, respectively. Data on the population size were obtained from DAFC[12]. The estimated number of doses per pig per year was calculated for each antibacterial and the following cut-offs were used: high (≥3.0), medium (≥1.0 and <3.0), low (≥0.5 and <1.0), very low (≥0.1 and <0.5) and negligible (<0.1). Weaners were excluded from the analysis as these were not found to present a potential risk for consumers. Use in piglets cannot be differentiated from use in sows since the use in these two groups is reported together. Available knowledge on the pharmacology of the most commonly used classes of antibacterials was also investigated;

3) Exposure assessment – the probability of human exposure to antibacterial residues in Danish pork was evaluated based on residue surveillance data from 2005-2009, from sows and slaughter pigs, respectively. The maximum prevalence of residues found above the MRLs was calculated for each antibacterial class and the following cut-offs were used (%): high (≥1.00), medium (≥0.50 and <1.00), low (≥0.10 and <0.50), very low (≥0.01 and <0.10) and negligible (<0.01);

4) Consequence assessment – the potential public health consequences of antibacterial residues in Danish pork and likelihood of its occurrence were described based on a

[12]Danish Agricultural and Food Council. Statistics 2008.

literature search. The literature search was conducted in May 2010 to identify reported cases of adverse reactions to antibacterial residues in meat products via PubMed database (National Center for Biotechnology Information, U.S. National Library of Medicine). Abstracts obtained were screened to ensure they were original reports of adverse reactions to antibacterial residues in meat products.

The steps 1-4 were combined into a risk estimate, where the outcome of each step was expressed in the following qualitative terms: high (event occurs very often), medium (event occurs regularly), low (event is rare but the occurrence is possible), very low (event is very rare but it cannot be excluded) and negligible (event is so rare that is not worth considering) (adapted from World Organisation for Animal Health (2004)). Final risk estimates were obtained by combining the qualitative outcomes. Classification matrices used are presented in annex A.

2.3.2. Surveillance system performance

According to the World Organisation for Animal Health (OIE), surveillance can be defined as the “systematic ongoing collection, collation, and analysis of data, and the timely dissemination of information to those who need to know so that action can be taken”. According to Thurmond (2003) surveillance performance appraisal must generally take into account accuracy, precision, rapidity and efficiency in determining the status of interest in a population.

Surveillance system *accuracy* can be defined as the ability to correctly identify the status of interest and is critically influenced by the sampling process and the diagnostic process in place (sensitivity and specificity). *Precision* refers both to the repeatability and reproducibility of the diagnostic test itself as well as the sampling scheme repeatability. *Rapidity* relates to the temporal ability of the surveillance system to detect, communicate and implement control measures. Finally, *efficiency* includes all aspects related to cost-effectiveness.

In this study, we focused on the evaluation of the antibacterial residue surveillance accuracy in Danish pigs.

2.3.2.1. Surveillance system accuracy

Assuming that in a population n animals are randomly sampled and tested using a given diagnostic test, animals can then be classified according to the test outcomes, T+, T-, respectively test positive and test negative. Let D+ and D- denote the true status of interest, respectively presence or absence of antibacterial residues in kidney samples (> or ≤ MRLs, respectively). Sensitivity (Se = Prob(T+|D+)) and specificity (Sp = Prob(T-|D-)) are commonly used to describe the test performance for a given target population and can be used to estimate the apparent prevalence ($ap = Se \times p + (1 - Sp) \times (1 - p)$, where p represents the true prevalence of antibacterial residues). If the population size (N) is much larger than n, then the sampling distribution of y is approximately binomial: y ~ Bin (n, ap). However, since D+ and D- are not know, the predicted values are the features of interest since they express the probability that a given test result is accurately expressing the true status. Predicted values can be calculated using Bayes' Theorem, according to the following equations:

Positive Predicted Value $(PPV) = Se \times p/(Se \times p + (1 - Sp) \times (1 - p))$;

Negative Predicted Value $(NPV) = Sp \times (1 - p)/((1 - Se) \times p + Sp \times (1 - p))$.

Also of interest is the probability of a false negative ($1\text{-}NPV$) and a false positive result ($1\text{-}PPV$).

Sampling sensitivity ($SampSe$) can be defined as the ability to identify at least one true positive in a sample and it can be estimated based on the posterior distribution of screening test sensitivity, true prevalence and sample size:

$$SampSe = 1 - (1 - Se)^{p \times n}$$

2.3.2.2. Bayesian modelling

Bayesian modelling techniques were used to obtain posterior distributions of the parameters of interest, combining both prior information and data. Uncertainty about sensitivity and true antibacterial residue prevalence were modelled using independent beta distributions (dbeta (a, b), based on the most-likely (modal) prior value of the parameter and the 5th or 95th percentile for the parameter) or uniform distributions (dunif (min, max), based on the minimum and maximum value of the parameter).

True prevalence of antibacterial residues at confirmation in sows and slaughter pigs was estimated including official and own check antibacterial residue surveillance data from 2005-2009. Test sensitivity and specificity were kept constant for sows and slaughter pigs, from 2005-2009. Test sensitivity was assumed to be primarily influenced by the 4-plate screening test characteristics and hence, a beta distribution was used based on the authors' best guess (modal value of 0.90 and 5th percentile of 0.80). Specificity was assumed to be perfect as confirmatory methods include different chemical techniques with high detection capability, accuracy and precision which allow to precisely quantify and identify the specific antibacterial residue (De Brabander et al., 2009). Prior true prevalence in sows and slaughter pigs was assumed to be given by a beta distribution (modal value of 0.001 and 95th percentile of 0.01). Posterior estimates of true antibacterial residue prevalence were compared across the years using Fisher's exact test, for sows and slaughter pigs, respectively; the posterior relative risk of antibacterial residues between sows and slaughter pigs was computed.

To evaluate the current surveillance system accuracy, the 4-plate screening test sensitivity and specificity was simulated based on the authors' best guess (sensitivity: modal value of 0.90 and 5th percentile of 0.80; specificity: modal value of 0.95 and 5th percentile of 0.90). In this case, prior knowledge regarding true prevalence in sows and slaughter pigs was assumed to be given by uniform distributions, based on the estimates obtained in the previous model (sows: minimum value of 0.0006 and maximum of 0.004; slaughter pigs: minimum value of 0.0001 and maximum of 0.0002). Test sensitivity and specificity were assumed to be constant for sows and slaughter pigs. Surveillance data were included to increase the accuracy of the posterior estimates. The positive predicted value for sows and slaughter pigs at screening was estimated. Sampling sensitivity was estimated for slaughter pigs surveillance, for different sample sizes, using the same screening sensitivity and specificity priors previously described. Two different scenarios were evaluated: 1) assuming the average antibacterial residue prevalence in slaughter pigs in 2005-2009; 2) assuming an increased prevalence in slaughter pigs similar to the average prevalence observed in sows in 2005-2009.

Additionally, to evaluate the effect of using four different hypothetical screening tests, the apparent prevalence of antibacterial residues at screening and positive predicted values were calculated. Tests 1-4 simulated different hypothetical screening tests and hence no surveillance data were included. The following priors were used: test 1 (sensitivity = 0.90; specificity = 0.98), test 2 (sensitivity = 1.00; specificity = 0.98), test 3 (sensitivity = 0.90; specificity = 1.00) and test 4 (sensitivity = 1.00; specificity = 1.00).

Beta buster software was used to estimate the parameters of a beta distribution based on the mode and upper or lower percentile of a given distribution (available at http://www.epi.ucdavis.edu/diagnostictests/). OpenBUGS v. 3.0.8 was used to estimate the posterior mean estimates of the parameters of interest and the associated 95% credibility intervals distributions using Markov chain Monte Carlo simulation (Lunn et al., 2009). Due to autocorrelation, thinning to every 100th observation was performed. Posterior distributions were obtained based on 10,000 iterations after discarding a burn-in of 10,000 iterations. Sample size was evaluated by comparing the standard deviation and the Monte Carlo-error (the difference should be less than 5%). Convergence was assessed by checking plots of the sampled values as time series, as well as auto-correlation plots (Toft et al., 2007).

3. RESULTS

3.1. RISK ASSESSMENT

3.1.1. HAZARD IDENTIFICATION

3.1.1.1. List of potential residues that might be found in pork

The list of potential residues that might be found in pork includes antibacterials, other veterinary medicinal products (anthelmintics, anticoccidials, carbamates and pyrethroids, sedatives and non-steroidal anti-inflammatory drugs), contaminants (e.g. mycotoxins, dyes, chemical elements, organophosphorus compounds, organochlorine compounds), substances having an anabolic effect (e.g. steroids, β-agonists, stilbenes and antithyroid agents) and prohibited substances (e.g. chloramphenicol and nitrofurans).

3.1.1.2. Residues in Danish pork

Residue surveillance data were used to estimate the probability of potential residues being present in Danish pork. No residues of prohibited substances have ever been detected in Danish pork. No samples have ever tested positive above the MRLs either for substances having an anabolic effect or for other veterinary medicinal products. Under the official residue surveillance program, residues of anabolic substances (steroids) have been found, but below the MRLs, in castrated pigs. The hypothesis of illegal use was ruled out as no other samples from the same herds were found positive. It was assumed by official authorities that the finding was a result of an imperfect castration. Regarding contaminants, except in 1990 where one sample exceeded the MRLs for heavy metals, no other samples have tested positive since then.

Antibacterial residue surveillance in Danish pigs between 2005 and 2009 included annual testing of approximately 6,700 and 20,700 samples, from sows and slaughter pigs, respectively. These data show an apparent average prevalence varying between 0.10-0.22% and 0.00-0.01%, in sows and slaughter pigs, respectively (Table 1 and Table 2). Although at a low prevalence, it was therefore concluded that antibacterial residues in Danish pork could be considered a potential hazard for human health – at least in sows. The probability of occurrence of other potential residues in Danish pork was considered insignificant and was therefore excluded from the further analysis.

3.1.2. Release assessment – antibacterial consumption in Danish pigs

The estimated numbers of antibacterial doses used per sow and slaughter pig per year, respectively, in 2008 are presented in Table 3. In annex B, a brief review of the most commonly used antibacterials in the Danish pig production is presented.

In Denmark, since 1998-1999, antibacterial use is only allowed for treatment of disease and not for growth promotion or prophylactic treatment. Since 2003, consumption of tetracyclines in slaughter pigs has increased significantly. In 2008, tetracyclines accounted for the major class of antibacterials used in Danish pig production and represented approximately 37% of all antibacterials used in slaughter pigs. Contrary, before 2006, macrolides were the class of antibacterials most commonly used in slaughter pigs. Since 2001, pleuromutilins (primarily tiamulin) are also commonly used in slaughter pigs and sows/piglets. Consumption largely increased in 2008 compared to 2007 due to a price reduction of the product.

In sows and piglets, since 2001, β-lactamase sensitive penicillins are the major antibacterial class used. In line, sulfonamides+trimethoprim constitute the second most commonly used antibacterial class in this age group.

Table 3

Consumption of antibacterials given as estimated number of doses[1] per sow and slaughter pig per year, Denmark, 2008.

	Number of animal daily doses per year given per	
Antibacterial	Sow	Slaughter pig
Aminoglycosides	0.8	0.4
Amphenicols	0.0	0.0
Cephalosporins[2]	0.2	0.0
Fluoroquinolones	0.0	0.0
Lincosamide/spectinomycin combinations	0.6	1.2
Macrolides (primarily tylosin)	0.7	4.9
Penicillins (β-lactamase sensitive)	3.2	3.4
Penicillins (other)	1.3	0.8
Pleuromutilins (primarily tiamulin)	1.8	6.3
Sulfonamides/trimethoprim	3.0	0.1
Tetracyclines	1.5	8.0
Other	0.0	0.0

[1]Estimated number of doses was calculated by dividing the animal daily dose (ADD) by the population size for sows (*N* = 1,020,000) and slaughter pigs (*N* = 21,900,000), respectively.

[2]Cephalosporins are mainly used in piglets and hence the number of doses per sow is likely to be overestimated, since Vetstat combines sow and piglet consumption data.

3.1.3. Exposure assessment – prevalence of antibacterial residues in Danish pigs

From 2005-2009, out of the total number of samples tested for antibacterial residues, on average 0.15% and 0.01% tested positive in sows and slaughter pigs, respectively (Table 1 and Table 2). Overall, this indicates a very low prevalence of antibacterial residues in Danish pork, in particular in pork from slaughter pigs. Amongst the few residues found above the MRLs, β-lactamase sensitive penicillins (benzylpenicillin) and tetracyclines (doxycycline, oxytetracycline) were most commonly found.

3.1.4. Consequence assessment – Public health risk of antibacterial residues in pork

It is generally accepted that antibacterial residues in food do not increase consumers' risk of antibacterial resistance (European Agency for the evaluation of veterinary medicinal products, 1999a).

Human consumption of antibacterial drugs might be associated with allergic and toxic reactions and also with disturbance of the gastrointestinal flora decreasing resistance to pathogenic bacteria and increasing risk of transfer of resistance factors among bacteria (Nord, 1993; Pallasch, 1988). The likelihood and magnitude of adverse consequences depends on the dose and pharmacokinetic properties of a given antibacterial. Moreover, the dose required to stimulate a reaction depends on the individual sensitivity (Katz and Brady, 2000).

Penicillins present the highest allergy rates observed among commonly used drugs. Penicillin allergic reactions in the human population, following therapeutic administration, are well-documented and are reported to vary from 1%-10%, mainly including cutaneous reactions. Anaphylactic reactions are unusual among treated patients (<0.04%) (Idsøe et al., 1968). Patients with history of penicillin allergy also present increased hypersensitivity to cephalosporins, which mainly include cutaneous reactions (Lin, 1992). Allergic reactions to tetracyclines and macrolides are rare and are considered to be of no concern regarding residues in food (Baldo et al., 2001; Dewdney et al., 1991). Sulfonamide adverse reactions are rare and have been mainly associated with skin sensitization, even though few cases of acute liver injury, pulmonary reactions, and blood dyscrasia have also been reported (Choquet-Kastylevsky et al., 2002).

Some studies showed that cold storage does not seem to significantly reduce the level of biologically active antibacterial residues in meat (Rose et al., 1996), while others reported a decrease in tissue concentrations (Korsrud et al., 1996). Pork products are generally consumed cooked and therefore the effect of processing and cooking in reducing antibacterial residues concentration is important to take into account. Studies on the effect of cooking on antibacterial residues in meat showed significant reductions in residue concentration (Furusawa and Hanabusa, 2002; Rose et al., 1996; Rose et al., 1997). However, it has been suggested that penicillin degradation products formed during cooking might also act as determinants for hypersensitivity (Warrington et al., 2010).

According to the literature search, six reports describing allergic reactions to antibacterial residues allegedly present in meat have been published and will be briefly discussed.

1. The case of a butcher who ingested fresh pork from a pig which had been injected with penicillin 3 days before slaughter and developed an anaphylactic reaction is reported (Tschevschner, 1972). The symptoms declined after 3-4 hours due to treatment with corticosteroids. Penicillin residues in the meat were investigated, and it was revealed that the meat contained 0.31-0.45IU penicillin per g meat. Moreover, the butcher was known to be allergic to penicillin. Note: Symptoms were presented immediately after consumption and hence it has been suggested that the ingested dose contained more than 10IU (Katz and Brady, 2000). This is in agreement with EMA, which suggests that at least 6µg of penicillin are necessary to elicit an allergic reaction (European Agency for the evaluation of veterinary medicinal products, 2008). Accordingly, adequate MRLs have been established to protect consumer health (e.g., benzylpenicillin - 50µg/Kg; oxacillin - 300µg/Kg).

2. In an experimental study, nine penicillin-sensitive volunteers were exposed to raw pig meat containing penicillin (0.02-0.04µg/g). Two presented itching immediately after. (Lindemayr et al., 1981). Note: even though adverse reactions were reported following consumption of raw pork with penicillin residues, the low frequency and intensity of the reactions might suggest that presence of penicillin residues in heat-

treated meat is unlikely to elicit allergic reactions. Additionally, a blind study should have been conducted preventing bias from affecting the validity of the results;

3. Schwartz and Sher (1984) described the case of a 45-year old woman with previous history of anaphylactic reaction to oral penicillin therapy. After eating a “frozen meat dinner” the woman presented pruritus and dyspnea. Skin tests presented a positive reaction to penicillin. The meat was reported to contain penicillin residues. Note: penicillin residues in meat were not quantified and therefore no definitive conclusions can be drawn from this study;
4. Tinkelman and Bock (1984) reported the case of a 14-year old girl who presented four anaphylactic episodes after eating meat. Skin and passive transfer tests presented a positive reaction to streptomycin and therefore the meat was supposed to contain residues of streptomycin. Allergy to animal proteins was excluded. Note: residues in meat were not quantified and therefore no definitive conclusions can be drawn from this study;
5. The case of a 64-year old female with previous history of anaphylactic reactions is reported. Two anaphylactic reactions occurred after ingestion of pork and beef. Allergy to animal proteins was excluded. Skin prick tests presented a positive reaction to penicillin (Kanny et al., 1994). Note: penicillin residues in meat were not investigated and therefore no conclusions can be drawn from the study;
6. More recently, a case of a 51-year old man who experienced an anaphylactic shock after eating minced steak was reported. Skin prick tests to penicillin presented a positive reaction. Allergy to animal proteins was excluded (Raison-Peyron et al., 2001). Note: penicillin residues in meat were not detected and therefore no conclusions can be drawn from the study.

The few reported cases generally lack scientific evidences that can definitely prove the association between the clinical reactions and the presence of penicillin residues in meat. However, the cases indicate that highly sensitive individuals might present allergic reactions to penicillin residues present in meat (Dewdney et al., 1991). Primary sensitization by residues of penicillins in food products is very unlikely and it is accepted that, at the most, allergic reactions might occur in already sensitized individuals. In previous sensitized individuals, it is believed that at least 6µg of penicillin are required to trigger a reaction (European Agency for the evaluation of veterinary medicinal products, 2008). Additionally, the oral route is reported to have much lower sensitization potential compared to parenteral administration (Dewdney et al., 1991). Given the high prevalence of penicillin allergy in the general population, the low number of cases reported in the literature suggests that if present in pork, the concentration of penicillin residues will - in the vast majority of cases - be too low to cause an adverse reaction.

Therefore, if present in food, after processing, cooking and further digestion, antibacterial residues are expected in general to be at a too low concentration for posing adverse effects to human health unless in the extreme and few cases, where the person is very sensitive.

3.1.5. RISK ESTIMATION

Table 4 presents an overview of the risk assessment and the risk estimated for the most commonly used antibacterials in the Danish pig production.

Release assessment showed that there is a medium to high probability of release of penicillins, pleuromutilins, sulfonamides/trimethoprim and tetracyclines in sows and lincosamide/spectinomycin combinations, macrolides, β-lactamase sensitive penicillins and tetracyclines in slaughter pigs. However, exposure assessment showed that the prevalence of residues is very low to negligible in sows and negligible in slaughter pigs. Consequences associated with antibacterial residues in Danish pork were classified as negligible, except for penicillins (very low). Hence, given the very low to negligible exposure of humans (dose and frequency) and very low to negligible consequences, the human health risk associated with antibacterial residues in Danish pork was estimated to be low to negligible in sows and negligible in slaughter pigs.

Table 4

Qualitative risk assessment of the human health risk of antibacterial residues possible found in Danish pigs, 2005-2009

Antibacterial	Assessment of risk[1]							
	Release[2]		Exposure[3]		Consequences		Risk estimation	
	Sows	Slaughter pigs	Sows	Slaughter pigs	Sows	Slaughter pigs	Sows	Slaughter pigs
Aminoglycosides	Low	Very low	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Amphenicols	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Cephalosporins	Very low	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Fluoroquinolones	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Lincosamide/spectinomycin combinations	Low	Medium	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Macrolides (primarily tylosin)	Low	High	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Penicillins, β−lactamase sensitive	High	High	Low	Negligible	Very low	Very low	Low	Negligible
Penicillins, other	Medium	Low	Negligible	Negligible	Very low	Very low	Negligible	Negligible
Pleuromutilins (primarily tiamulin)	Medium	High	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Sulfonamides/trimethoprim	High	Vey low	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Tetracyclines	Medium	High	Very low	Negligible	Negligible	Negligible	Negligible	Negligible
Other	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible

[1]Qualitative risk terms: high (event occurs very often), medium (event occurs regularly), low (event is rare but the occurrence is possible), very low (event is very rare but it cannot be excluded) and negligible (event is so rare that is not worth considering).

[2]Probability of release was evaluated based on the estimated number of antibacterial doses per pig per year: high (≥ 3.0), medium (≥1.0 and <3.0), low (≥0.5 and <1.0), very low (≥0.1 and <0.5) and negligible (<0.1).

[3]Probability of exposure was evaluated based on the maximum proportion of antibacterial residues (%) above maximum residue limits: high (≥1.00), medium (≥0.50 and <1.00), low (≥0.10 and <0.50), very low (≥0.01 and <0.10) and negligible (<0.01).

3.2. Surveillance system accuracy

Table 5 presents the posterior estimates of true antibacterial residue prevalence at confirmation, from 2005-2009, for sows and slaughter pigs. Overall, results show that the prevalence of antibacterial residues in Danish pigs is very low. Still, antibacterial residue prevalence was found to be higher in sows compared to slaughter pigs, with the median relative risk ranging from 10-25 (data not shown). From 2005-2009, the antibacterial residue prevalence remained constant in sows and slaughter pigs, respectively.

Table 6 presents the posterior estimates of 4-plate method sensitivity and specificity. Results suggest that the 4-plate method sensitivity is, on average, 88% and specificity is very high (>99%). The positive predicted value obtained for sows and slaughter pigs, in 2008, was 95.60% (91.94-98.16%) and 53.79% (32.95-77.61%), respectively.

Table 7 present the posterior estimates of the current sampling sensitivity (i.e., the probability of detecting at least one true positive) for different sample sizes in slaughter pigs, using the 4-plate method, assuming different antibacterial residue prevalence scenarios. Under the current antibacterial residue prevalence (scenario 1), sampling sensitivity is significantly reduced from approximately 100% to 95% when sample size is reduced from 20,000 to 10,000 samples. When the true prevalence in slaughter pigs was increased to the prevalence observed in sows (scenario 2), the sampling sensitivity was significantly higher. Accordingly, in scenario 2, even when sample size was reduced to 5,000 samples, sampling sensitivity was still very high (100%).

Table 8 shows the impact of different hypothetical screening tests on the apparent prevalence and the positive predicted value. Results obtained for test 1 and test 2 show that the higher the prevalence, the higher the positive predicted value, which is in accordance with basic rules of testing. Accordingly, for test 1 and test 2 the positive predicted value was higher in sows compared to slaughter pigs. Improvement of test sensitivity from 90% to 100% only resulted in a slightly increase of the apparent prevalence and the positive predicted value (test 2 vs. test 1). When test specificity was assumed to be perfect (test 3 and test 4), the positive predicted value increased to 100% and hence, the apparent prevalence was lower as the number of false positives was reduced to zero.

Table 5

Posterior mean estimates and 95% credibility intervals of true antibacterial residue prevalence obtained by Bayesian analysis, using informative priors for test sensitivity[1] and specificity[2] and assuming a common true prevalence in Danish sows and slaughter pigs[3], 2005-2009

Age group	Year	True antibacterial residue prevalence (%)
Sows	2005	0.16 (0.08-0.28)
	2006	0.15 (0.07-0.28)
	2007	0.13 (0.06-0.23)
	2008	0.26 (0.15-0.40)
	2009	0.23 (0.12-0.36)
Slaughter pigs	2005	0.01 (0.00-0.03)
	2006	0.01 (0.00-0.04)
	2007	0.02 (0.00-0.04)
	2008	0.02 (0.00-0.04)
	2009	0.01 (0.00-0.03)

[1]Prior test sensitivity given by *Se* ~ dbeta (45.6, 5.6), modal value = 0.90, 5th percentile = 0.80.

[2]Prior test specificity given by *Sp* = 1.

[3]Prior true prevalence given by *p* ~ dbeta (1.4, 364.9), modal value = 0.001; 95th percentile = 0.01.

Posterior estimates for test sensitivity: 86.33 (74.30-95.02).

Table 6

Posterior mean estimates and 95% credibility intervals of the 4-plate method sensitivity and specificity obtained by Bayesian analysis

Priors		Posterior estimates (%)
Sensitivity[1]	modal value = 0.90; 5th percentile = 0.80	87.7 (78.8-95.4)
Specificity[2]	modal value = 0.95; 5th percentile = 0.90	99.9 (99.9-99.9)

[1]Prior test sensitivity Se ~dbeta (45.6, 5.6).

[2]Prior test specificity Sp ~ dbeta (99.7, 6.2).

Table 7

Posterior mean estimates of sampling sensitivity (defined as the probability of detecting at least one pig with antibacterial residues above the MRLs) obtained by Bayesian analysis, for different sample sizes in Danish slaughter pigs, using the current 4-plate screening method. Two scenarios were modelled: 1) assuming the true antibacterial residue prevalence in slaughter pigs[1]; 2) assuming an increased prevalence in slaughter pigs similar to the true antibacterial residue prevalence observed in sows[2]

Scenarios	Sample size	Sampling sensitivity (%)
1 - Prevalence in slaughter pigs	20,000	99.6
	15,000	98.6
	10,000	95.1
	5,000	80.3
	1,000	29.8
2 - Higher prevalence (prevalence in sows)	20,000	100.0
	15,000	100.0
	10,000	100.0
	5,000	99.9
	1,000	88.9

[1]Scenario 1: prior prevalence in slaughter pigs given by $p1_{slaughter\ pigs}$ ~ dunif (0.0001, 0.0004).

[2]Scenario 2: prior prevalence in slaughter pigs given by $p2_{slaughter\ pigs}$ ~ dunif (0.0006, 0.0040).

The 4-plate test characteristics can be found in Table 6.

Table 8

Posterior mean estimates and 95% credibility intervals of apparent prevalence of antibacterial residues at screening and positive predicted value obtained by Bayesian analysis for different hypothetical tests, assuming different true prevalences in Danish sows[1] and slaughter pigs[2].

Test	Priors	Apparent prevalence (%)		Positive predicted value (%)	
		Sows	Slaughter pigs	Sows	Slaughter pigs
Test 1 (default test)	*Se* = 0.90 *Sp* = 0.98	2.27 (2.17-2.35)	2.01 (2.01-2.03)	12.25 (8.10-15.13)	0.72 (0.46-1.39)
Test 2 (perfect sensitivity)	*Se* = 1.00 *Sp* = 0.98	2.30 (2.19-2.35)	2.02 (2.01-2.03)	13.42 (8.92-16.53)	0.80 (0.51-1.54)
Test 3 (perfect specificity)	*Se* = 0.90 *Sp* = 1.00	0.28 (0.18-0.36)	0.02 (0.01-0.03)	100.00	100.00
Test 4 (perfect test)	*Se* = 1.00 *Sp* = 1.00	0.31 (0.20-0.40)	0.02 (0.01-0.03)	100.00	100.00

[1]Prior prevalence in sows given by p_{sows} ~ dunif (0.0006, 0.0040).

[2]Prior prevalence in slaughter pigs given by $p_{slaughter\ pigs}$ ~ dunif (0.0001, 0.0004).

4. Discussion

4.1. Risk assessment

In this study, a qualitative risk assessment approach was used. For transparency purposes, criteria and terms used were clearly defined and data were extensively documented.

It is generally accepted that "zero risk" is impossible to achieve in the context of food safety (Food and Agriculture Organization of the United Nations, 1998). However, in this study the definition of negligible could not be differentiated from zero and hence, according to the classification matrix used, the product of negligible probability implied that the risk was negligible, indicating that the event was "so rare that it was not worth considering" (annex A).

Antibacterial residue surveillance in Danish pigs includes both official and own check surveillance which assures a high sampling level (0.1% of the total slaughter pig population and more than 1% of the sows slaughtered in the previous year), exceeding the 0.03% level required by EU authorities. Antibacterial residue prevalence in Danish pigs has been consistently very low and no changes were observed after the ban of the antibacterial growth promoters in 1998-1999. Besides antibacterial residue surveillance activities, Denmark has other requirements regarding use of antibacterials in the pig production, which further contributes to mitigate the human health risk of antibacterial residues in pork. Since 2000, antibacterial use data are routinely collected including information on target animal species, and age group of animals treated – Vetstat (Stege et al., 2003). Additionally, since 2005, DVFA publishes guidelines for antibacterial treatment of food-producing animals. These guidelines provide veterinarians with a working tool focused on prudent antibacterial usage, efficacy of treatment of animals, low development of resistance and public health concerns (National Food Institute and Statens Serum Institut, 2009). Furthermore, the industry has extended the withdrawal period for tetracyclines to 30 days and banned the use of sulfadimidine in sows and slaughter pigs. For a further discussion on the extension of the withdrawal period for tetracyclines, please refer to annex B ("tetracyclines"). Regardless of the substance used, withdrawal periods of less than 5 days are not accepted[13]. Additionally, fluoroquinolones are not used in Danish livestock and consumption of cephalosporins is very low in Danish pigs.

At the EU level, very conservative assumptions, as illustrated by the current standard food basket, are used for determining ADIs, MRLs and withdrawal times, assuring a very high level of protection to consumers. Accordingly, it has been shown that when MRLs for tetracyclines in meat are exceeded by a factor of 400, the risk of an adverse reaction in humans is estimated to be 1 in 3 millions exposed consumers (Berends et al., 2001).

Reported cases of allergic reactions to penicillin residues in food are mainly attributed to milk, and mostly occurred in the 1960s where no antibacterial residue surveillance and control programs were in place in most countries (Dayan, 1993). Additionally, according to a

[13] Danish Crown. Code of practice. Rules for Danish Crown's pig farming co-operative members/suppliers.

published review on the association of penicillin in milk and hypersensitivity, reported cases are generally poorly documented and fail to prove a causal relationship (Dewdney and Edwards, 1984). Still, even when present at very low concentrations, penicillin residues in milk might cause allergic reactions in highly sensitive individuals (Borrie and Barrett, 1961; Ormerod et al., 1987). Despite the lack of well-documented cases of allergic reactions due to penicillin residues in meat, we have considered that hypersensitive individuals might present adverse responses to very low doses of penicillin residues, if present in Danish pork.

Overall, risk assessment results showed that the human health risk associated with antibacterial residues in Danish pork are low to negligible in sows and negligible in slaughter pigs. This is in agreement with previous studies assessing the human health risk of antibacterial residues in food products (Berends et al., 2001; Dayan, 1993; Dewdney et al., 1991; Waltner-Towes and McEwen, 1994; Woodward, 1991). Accordingly, in the Netherlands, the human health risk associated with presence of tetracycline residues was estimated to be 80,000 times lower than the risk of human salmonellosis through pork products (Berends et al., 2001). Moreover, the small concentrations of antibacterial residues through which humans are exposed to through food represent a negligible proportion of the total amount of antibacterials consumed by humans (Cerniglia and Kotarski, 2005). Hence, long-term effects are also not likely to be expected from consumption of Danish pork as human exposure to antibacterial residues is very low to negligible and below the ADI for lifetime exposure. This is in agreement with previous studies (Paige et al., 1997). Additionally, the number of sows represents only a minor proportion of the total pork consumed in Denmark, which further reduces the overall risk posed to Danish consumers.

A qualitative risk assessment must be undertaken, before a quantitative approach is used and this might be used to answer a specific question, identifying the data required, the risk pathway, and risk management options. Furthermore, a qualitative risk assessment enables the risk manager to judge whether a quantitative risk assessment is subsequently necessary (Anon., 2009; Vose et al., 2001). The qualitative risk assessment presented here has shown to be a useful tool to address the question of interest, providing a transparent and well-documented evaluation of the human health risk associated with residues in Danish pork, without requiring a further quantitative assessment. Available data were included wherever possible and subjectivity was kept to a minimum. Results presented contribute to the decision-making process and sustain the need to further evaluate a risk-based approach to antibacterial residue surveillance, improving the overall cost-effectiveness of the Danish residue surveillance program in pigs.

According to EU legislation, residue surveillance aims at assuring the protection of human health from potential residues in food of animal origin. Although there are strict regulations to assure food safety along the food chain, available data indicate that European consumers perceive the risk of residues in food as one of the most important concerns amongst food borne risks (European Commision, 2006). Hence, residue surveillance is also important for public perception of activities regarding food safety. However, reasons other than food safety might apply and require that residue surveillance activities are in place. In line, residue data might be used as an indicator of animal health and welfare, use of veterinary drugs, meat quality and compliance with regulations. Above all, residue surveillance might be required to document fulfillment of export requirements to countries outside the EU.

4.2. SURVEILLANCE SYSTEM ACCURACY

Bayesian modelling techniques can be used to estimate a certain parameter combining both prior information and data to obtain a posterior distribution of the parameter of interest. With the advent of powerful software allowing for using Markov chain Monte Carlo sampling algorithm, Bayesian methods have been increasingly used in veterinary medicine for the evaluation of diagnostic tests in the absence of a gold standard (Branscum et al., 2005; Georgiadis et al., 2003). Still, to our knowledge no studies were conducted on the use of Bayesian methods to evaluate the accuracy of a surveillance system.

Overall, true antibacterial residue prevalence in Danish pork was very low. Still, true antibacterial residue prevalence was found to be relatively higher in sows compared to slaughter pigs (Table 5). This might be explained by different practices regarding antibacterial use and management amongst sows and slaughter pigs. From 2005-2009, the most common causes of positive findings were found to be related to poor keeping of treatment records and/or inadequate identification of treated animals (data not shown). These "mistakes" are more likely to occur in sows, where individual management is used (against batch management in slaughter pigs) and where the time of slaughter is not known. To further reduce the low prevalence of residues in Danish sows, own-check farm programs should be followed, as enforced by Danish legislation[14], including good management practices regarding antibacterial use and compliance with withdrawal periods. Education of farmers and farm workers should also be promoted to increase awareness regarding the impact of potential detection of antibacterial residues above the MRLs on industry reputation and above all, exports.

4.2.1. Screening methods

In the context of antibacterial residue testing, analytical sensitivity refers to the lowest concentration of a certain residue a test can detect, i.e., detection capability; analytical specificity indicates the ability of a test to react only to one antibacterial residue (Dohoo et al., 2003). According to EU legislation, screening methods should be validated and have a detection capability with an error probability <5%. Epidemiological sensitivity and specificity (defined in the material and methods section) are related to, but differ from analytical sensitivity and specificity. Prior estimates of screening test sensitivity and specificity should

[14] Bekendtgørelse om dyreejeres anvendelse af lægemidler til dyr samt offentlig kontrol og fødevarevirksomheders egenkontrol med restkoncentrationer. BEK nr. 780 af 31/06/2010.

be obtained from published studies or expert opinion, as appropriate. However, due to lack of data and adequate studies to evaluate diagnostic test characteristics, test sensitivity and specificity priors were based on the authors' best guess. Overall, study results were used to illustrate the impact of different hypothetical screening tests on surveillance system accuracy.

When evaluating the use of an alternative screening test, various aspects should be carefully considered. Screening methods are required to have high sensitivity and specificity and to provide results in reduced time, at low running costs. Sample preparation and testing procedures must be as simple as possible and must not require expensive equipment. Suspect samples detected at screening are further sent either to post-screening or directly to confirmation. Hence, the overall surveillance system accuracy and costs are strongly influenced by the screening test characteristics. Confirmatory tests should present very high specificity, but they are generally laborious and time-consuming, require expensive equipments, high technical expertise and hence, cannot be applied to a large number of samples.

Results obtained for the current 4-plate method suggest that posterior estimates of test sensitivity ranged from 79% to 95% and specificity was estimated to be very high (>99%) (Table 6). In sows, the positive predicted value posterior estimate was high (>90%). However, in slaughter pigs, due to the low prevalence of antibacterial residues, posterior estimates ranged from 33% to 78%, suggesting the need to further investigate a different strategy aiming at reducing the proportion of false positive results (e.g., risk-based surveillance approach). The 4-plate method currently in use in Denmark allows obtaining a result in approximately 24 hours (48 hours in case of a positive finding due to retesting) and is assumed to produce a low number of false positive test results. However, some antibacterials including aminoglycosides (e.g. dihydrostreptomycin, spectinomycin), quinolones (e.g. ciprofloxacin, marbofloxacin, oxolinic acid), macrolides (e.g. tylosin) and sulfonamides (e.g. sulfadiazine) are not likely to be detected using this method (Birgitte Herbert Nielsen, personal communication). However, from a public health perspective, these are not likely to represent the most relevant antibacterial residues, as it will be discussed following. Aminoglycoside consumption is low in Danish sows and very low in slaughter pigs, and they are mainly excreted unchanged, via urine. However, aminoglycosides tend to be bound to the renal cortex where detectable residues can persist and hence further studies should be conducted to assure compliance with MRLs. Still, because of the low oral bioavailability, residues in food are not of concern regarding human food safety (Gehring et al., 2005). Regarding fluoroquinolones, these are almost not used in Danish pigs (8g in total in 2008) and therefore are not likely to be found in Danish pork. Regarding tylosin, which is the macrolide most commonly used in slaughter pigs to treat *Lawsonia intracellularis* related infections and to a lesser extent, respiratory diseases, residue depletion studies showed that residues are generally low and deplete rapidly. This suggests that tylosin residues are not expected to be a relevant hazard in Danish pork. Finally, to overcome some of the 4-plate method limitations, each year, DVFA tests samples for sulfonamides using chemical methods and no positives have ever been found, suggesting that this is not a critical drug residue in Danish pork. Moreover, consumption of sulfonamides in slaughter pigs is very low and use of sulfadimidine in sows is banned since 1990. Regarding pleuromutilins, because tiamulin residue concentrations in kidney are considered to be too low to be detected, chemical methods are also used by DVFA to investigate the presence of residues in matrices other than kidney and no positives have ever been detected.

Results obtained for the four different hypothetical screening tests show the effect of test sensitivity and specificity on the apparent prevalence and positive predicted value. Our model results show that improvement of test sensitivity alone (test 2 vs. test 1) resulted in a slightly increased apparent prevalence and positive predicted value. Still, the positive predicted value was very low (<16% and <2% in sows and slaughter pigs, respectively), indicating a very high proportion of false positive results. Three different approaches might be considered to increase the positive predicted value (Dohoo et al., 2003). It can be achieved by sampling animals where prevalence is expected to be high, i.e., targeting sampling to high risk-animals. A second approach considers the use of a test with higher specificity and with the same or higher sensitivity, which we have shown by using test 3 and test 4. Finally, a multiple test approach can also be considered, which could include the use of an additional post-screening test.

Model results show that screening tests should ideally be highly sensitive and highly specific. However this might not be technically and economically feasible. Hence, an alternative option could include a screening test with very high sensitivity combined with a post-screening test with very high specificity, reducing the number of samples further sent for confirmation. Overall, results obtained clearly indicate that screening tests strongly impact the surveillance system accuracy and hence, further studies should be conducted to obtain correct information regarding sensitivity and specificity estimates of other relevant alternative tests.

4.2.1.1. Nouws antibiotic test

Different screening and post-screening tests are available, using different matrices and procedures (De Brabander et al., 2009; Pikkemaat et al., 2008b). Currently, the most relevant alternative to the 4-plate method is the Nouws antibiotic test (NAT) (Birgitte Herbert Nielsen, personal communication).

NAT has been validated according to EU requirements and is used for screening antibacterial residues in the Netherlands since 2004. NAT uses renal pelvis fluid juice and five plates (T – tetracycline; Q – quinolones; B&M – β-lactams and macrolides, including lincosamides and pleuromutilins; A – aminoglycosides; S – sulfonamides). Samples are applied into punch holes and incubated at appropriate temperatures for 16–18 hours (Pikkemaat et al., 2008b). NAT allows detecting residues at very low concentrations, below the MRLs, which results in a high number of suspect samples that must be further tested using a post-screening method before chemical testing is conducted. Moreover, no MRLs have been established to renal pelvis fluid and hence a post-screening test in approved matrices is required.

Published data from the Netherlands strongly indicate that NAT yields a large number of false positives. In a study conducted on 444 suspect samples, 67 samples were detected as suspect at screening. At post-screening on kidney, 46 were identified as suspect and finally, at confirmation only 5 were further found to be above the MRLs (Pikkemaat et al., 2009).

In the Netherlands, due to the low number of positive samples at screening for macrolides, β-lactams, sulfonamides and quinolones, suspect samples at initial screening are directly sent for confirmation.

The post-screening test uses four plates (T, Q, B&M and A), allowing for the identification of a specific antibacterial group after incubation at appropriate temperatures for 16–18 hours. The NAT post-screening method uses kidney extract obtained after homogenization and centrifugation and can be limited to the positive test plate. Aminoglycoside and tetracycline suspect samples are further sent to post-screening. At post-screening, tetracyclines are tested along with a quality control (kidney fortified with 600μg/kg of oxytetracycline); when the inhibition zone is larger than the control, the sample is forwarded for confirmation. Currently, aminoglycosides confirmatory analysis is performed on all positive post-screening samples, but a similar approach as used for tetracycline post-screening including the use of a quality control is going to be introduced (Mariel Pikkemaat, personal communication). Figure 3 presents a schematic representation of the NAT and post-screening method. To a further discussion on the Dutch antibacterial residue surveillance scheme, please refer to annex C.

Regarding sample preparation, NAT includes a simple and less laborious technique, compared to the 4-plate method (Pikkemaat et al., 2008a). Moreover, renal pelvis fluid is used, where antibiotic concentration is higher and natural growth inhibiting factors are lower, compared to the kidney tissue (Nouws, 1981). Compared to the 4-plate method currently used for screening antibacterial residues in Denmark, the NAT presents a higher detection capability and is able to detect residues of aminoglycosides (e.g. dihydrostreptomycin), quinolones (e.g. marbofloxacin and oxolinic acid) and some sulfonamides below or at the MRLs, which are not likely to be detected with the 4-plate method. Additionally, published results indicate that NAT detects tetracycline residues at lower concentrations than with the 4-plate method, at least at half the MRLs (Pikkemaat et al., 2008a). This suggests that NAT might be useful to identify slaughter pigs presenting residues below the MRLs, which should not be exported to countries where lower tolerance limits apply. Table 8 presents a comparison between the 4-plate method and the NAT.

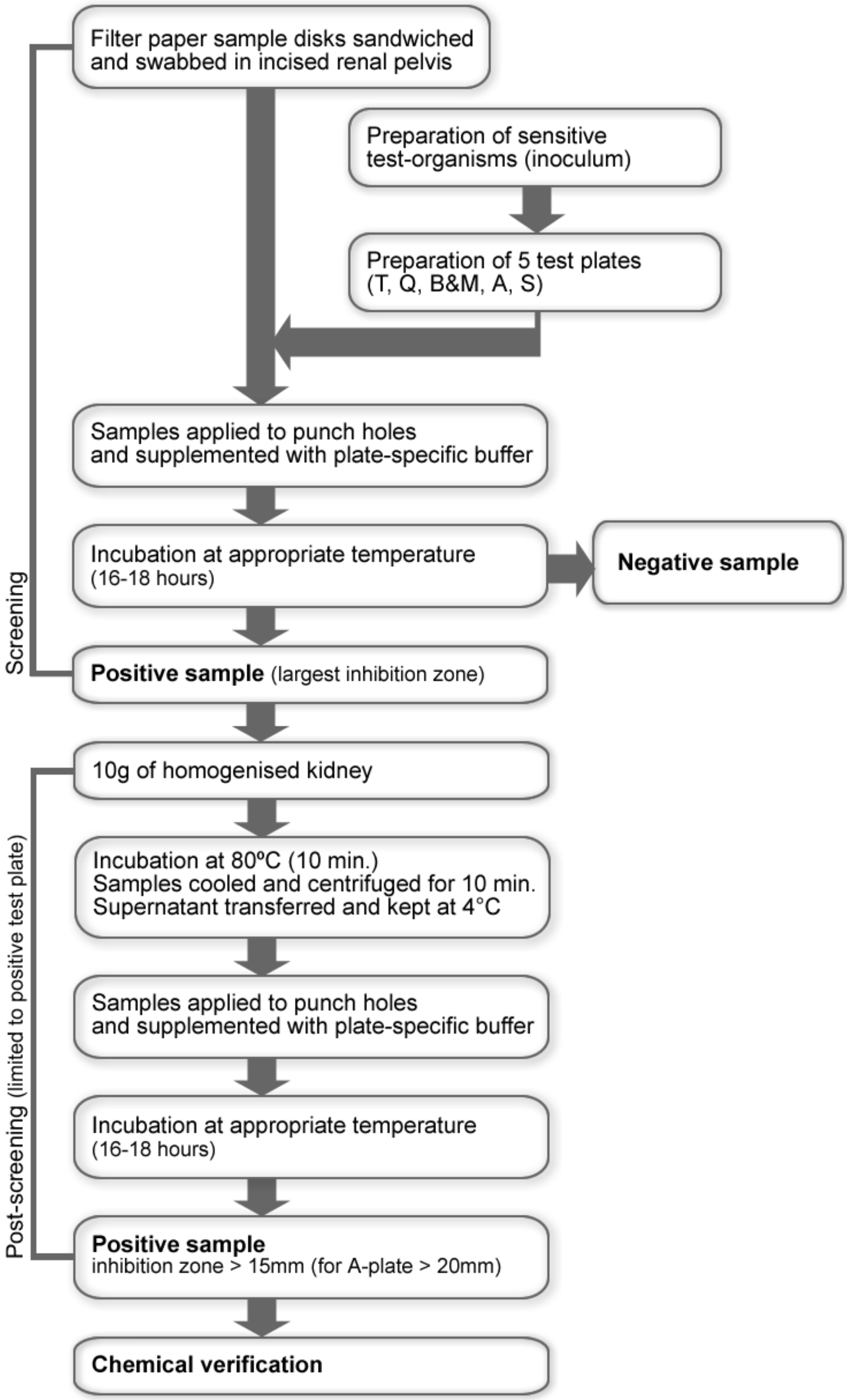

Fig. 3. Schematic representation of the Nouws antibiotic test (adapted from Mariel Pikkemaat).

Table 8

Main differences between the 4-plate method and the Nouws antibiotic test

Screening method	4-plate	Nouws antibiotic test
Matrix	Kidney tissue	Renal pelvis fluid juice
Plates	4 plates: - pH 6: *Bacilus subtilis* - pH 7 + t: *Bacilus subtilis* + trimethoprim-solution - pH 8: *Bacilus subtilis* - pH 8M: *Kocuria rhizophila* (formerly *Micrococcus luteus)*	5 plates: - T: *Bacilus cereus* - Q: *Yersinia ruckeri* - B&M: *Kocuria rhizophila* - A: *Bacilus subtilis* - S: *Bacilus pumilus*
Method	Discs of tissue are plated and incubated (5 discs per plate)	Filter paper sample disks impregnated with renal pelvis fluid are applied to punch holes, supplemented with plate-specific buffer and incubated (34 discs per plate)
Time (incubation)	20-24h In case of a positive finding the sample is retested (20-24h)	16-18h In case of a positive finding in the T- or A-plate, post-screening is conducted (16-18h)
Post-screening	-	4 plates: - NK-T: *Bacilus cereus* - NK-Q: *Yersinia ruckeri* - NK-B&M-plate: *Kocuria rhizophila* - NK-A: *Bacilus pumilus*
Confirmation	12-plate method + chemical analysis	Chemical analysis
Pros	- Low number false positive results - No licensing method	- No licensing method - High detection capability for tetracyclines and aminoglycoside - Higher antibacterial concentration in renal pelvis fluid than kidney - Fewer natural growth inhibiting factors in renal pelvis fluid - Punch holes improves diffusion process
Cons	- Cannot verify compliance with MRLs for all antibacterials - Higher concentration of natural growth inhibiting factors in kidney	- Cannot verify compliance with MRLs for all antibacterials - High number false positive results

4.2.2. Risk-based approach to antibacterial residue surveillance in slaughter pigs

Antibacterial residue prevalence in Danish slaughter pigs showed no significant difference between the years 2005-2009 and was found to be negligible. Given the true antibacterial prevalence found in slaughter pigs, for a sample size of 20,000 slaughter pigs, on average, only one to two samples are expected to present residues above the MRLs. As it resembled a “rare prevalence” scenario, it was decided to evaluate the sampling sensitivity (i.e., the ability of the system to detect at least one sample presenting residues above the MRLs) amongst slaughter pigs. Moreover, given that the aim of the surveillance program is broader than to ensure detection of a positive pig, it is important to identify which herds might potentially present a higher likelihood of non-compliance with antibacterial use requirements.

Two different scenarios were used, for illustration purposes. Scenario 1 simulated the current antibacterial residue prevalence found in Danish slaughter pigs, in 2005-2009, whereas scenario 2 illustrated a risk-based scenario, where the prevalence of antibacterial residues in slaughter pigs was increased to the same level as in sows.

For the current prevalence scenario and sample size (20,000 samples), the sampling sensitivity was very high (>99%). However, when sample size was reduced to 1,000 samples, sampling sensitivity was reduced to 29%, suggesting that it was very unlikely to detect a sample presenting residues above the MRLs. This is in agreement with official residue surveillance data, where, on average, 890 samples were collected each year and, from 2005-2009, no residues were found above the MRLs. However, scenario 2 suggests that, if sampling is targeted to high-risk slaughter pigs, sampling sensitivity can be significantly increased, even when the sample size is reduced to 5,000 samples.

Overall, results show that sampling sensitivity increases with: increased prevalence; increased test sensitivity; and/or increased sample size. However, it was also shown that for the same or reduced sample size, the sampling sensitivity can be highly increased when targeted sampling is performed, instead of random sampling. This strongly indicates that a risk-based approach to antibacterial residue surveillance in slaughter pigs should be further investigated, targeting sampling to high-risk animals and increasing the ability to detect a true positive slaughter pig. High-risk herds might be identified based on antibacterial use data (e.g. higher or lower use than average) and post-mortem meat inspection data (e.g. herds systematically presenting pigs with pulmonary lesions at slaughter are more likely to have higher use of antibacterials). The risk-based and the non-risk based surveillance component should then be combined, so that an overall estimate of the surveillance system accuracy can be estimated, while adjusting for the higher relative risk in the risk-based population (Hadorn and Stark, 2008).

5. CONCLUSION

Since 1972, no residues of prohibited substances or residues of substances above the MRLs have been detected in Danish pork, except for antibacterials. It was therefore considered that the human health risk from residues other than antibacterials in Danish pork was negligible. The human health risk associated with antibacterial residues in Danish pork was estimated to be low to negligible in sows (with the low risk being associated with penicillin residues) and negligible in slaughter pigs. However, reasons other than food safety might also apply and require that surveillance activities are in place. These include public perception of animal health and welfare, and more importantly, documentation of compliance with regulations and export requirements to countries outside the EU.

A Bayesian model was developed to evaluate the Danish antibacterial residue surveillance system accuracy and the consequences of introducing alternative sampling schemes and screening tests. Model results show that use of alternative screening tests significantly impact surveillance system accuracy. Results obtained also suggest that the current screening method used in Denmark presents high sensitivity and very high specificity for detecting residues below or at the MRLs, for most of the antibacterials of interest. However, if importing countries require compliance with lower limits, alternative screening tests should be considered. Further studies should include a throughout evaluation of alternative screening tests sensitivity and specificity, as well as other test characteristics including time, costs and complexity of testing procedure.

Overall, true antibacterial residue prevalence in Danish pigs is very low. Compared to sows, slaughter pigs present negligible, 10-25 times lower antibacterial residue prevalence. The effect of a risk-based surveillance approach in slaughter pigs was therefore evaluated. Model results suggest that, if high-risk slaughter pigs can be identified, the number of samples from slaughter pigs can be largely reduced while increasing the sampling sensitivity (defined as the probability of detecting at least one slaughter pig presenting residues above the MRLs).

The most common causes of the few samples presenting residues above the MRLs were related with non-compliance with withdrawal periods, generally by mistake, due to inappropriate identification of treated sows. Strict compliance with own check requirements already enforced by Danish legislation could be promoted to further reduce antibacterial residue prevalence in Danish pigs, particularly in sows.

6. PERSPECTIVES

EU legislation allows that each country defines the screening and confirmatory methods used, the minimum number of tests that should be performed by sample, which drugs should be analysed and sampling criteria which provides a flexible framework that can be adjusted to each country's specific context. Moreover, according to EU requirements residue surveillance programs should be risk-based, targeting sampling to high-risk animals.

To both comply with EU and export requirements and increase the cost-effectiveness of the current residue surveillance program in Danish pigs, further specific studies are required. These should include an evaluation of alternative screening and post-screening test characteristics and investigation of a risk-based approach for residue surveillance.

6.1. Risk mitigation strategies

Even though the overall antibacterial residue prevalence in Danish pigs is very low, sows present 10-25 times higher risk of presenting antibacterial residues above the MRLs, compared to slaughter pigs. This suggests that actions should be taken to further reduce the prevalence of antibacterial residues in Danish sows. These should include strict compliance with own check farm requirements regarding antibacterial use, as already enforced by Danish legislation. Special focus should be given to the appropriate record keeping systems and identification of treated sows to reduce the number of sows delivered to slaughter before the end of the withdrawal period.

6.2. Screening methods

Use of alternative screening tests significantly impact surveillance system performance and costs. However, data is lacking to perform a throughout evaluation of the epidemiological and economic consequences of such changes.

Currently, the most relevant alternative to the current screening method used in Denmark is NAT, which is used in the Dutch residue surveillance program, since 2004. Hence, adequate studies should be conducted to evaluate NAT under the Danish scenario, where antibacterial use and withdrawals periods might differ from the Dutch situation, which is likely to impact its performance. Further research should focus on the throughout evaluation of NAT and post-screening test sensitivity and specificity as well as other test characteristics including time, costs and complexity of testing procedure.

6.3. Confirmatory methods

Regarding residue confirmation, the development of multi-class LC-MS methods for simultaneous identification and quantification of several antibacterials points this as one of the preferred methods for antibacterial residue confirmation in the future. LC-MS methods are expected to increase detection capability and reduce the time required for confirmation results (Gitte Geertsen, personal communication). Further research should focus on the evaluation of alternative cost-effective chemical methods with high throughput, allowing for the identification of several antibacterials in the same sample.

6.4. Risk-based surveillance

According to EU requirements, national residue surveillance plans should be targeted to high-risk animals, accounting for different risk factors including sex, age, fattening system or any evidence of misuse of substances. The risk-based surveillance framework might be used to evaluate different surveillance strategies aiming at improving surveillance system cost-effectiveness and therefore increasing the probability of detecting a pig presenting residues above the MRLs, while keeping expenses to a minimum.

Risk-based methods for antibacterial residue surveillance in Danish pigs should be further evaluated including information on post-mortem meat inspection and antibacterial use. Use of antibacterial use data would be possible in Denmark because the Vetstat database contains data describing use of antibacterials by animal species and age group.

To avoid negative impact of an eventual higher prevalence found in a risk-based sample, an investigation of the effect of a risk-based approach should be studied, separately from the current non risk-based antibacterial residue surveillance. Further studies should then carefully evaluate how to integrate data from risk-based and non risk-based surveillance.

6.5. Other animal species and animal products

Surveillance results from other food-producing species including cattle, sheep, goats and horses and animal products (milk) indicate that antibacterial residue prevalence is negligible. Furthermore, the 4-plate method is also used for screening antibacterial residues in these animal species.

Diagnostic test evaluation and risk-based surveillance studies might be extended to other food-producing species and animal products aiming at improving the overall cost-effectiveness of residue surveillance in Denmark.

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ANNEX A

RISK ESTIMATION MATRICES

Release x Exposure

	Exposure				
Release	Negligible	Very low	Low	Medium	High
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Very low	Negligible	Very low	Low	Low	Low
Low	Negligible	Low	Low	Medium	Medium
Medium	Negligible	Low	Medium	Medium	High
High	Negligible	Low	Medium	High	High

(Release x exposure) x consequences

	Consequences				
Release x Exposure	Negligible	Very low	Low	Medium	High
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Very low	Negligible	Very low	Low	Low	Low
Low	Negligible	Low	Low	Medium	Medium
Medium	Negligible	Low	Medium	Medium	High
High	Negligible	Low	Medium	High	High

ANNEX B

BRIEF REVIEW OF THE MOST COMMONLY USED ANTIBACTERIALS IN DANISH PIGS, 2005-2009

Tetracyclines

Tetracyclines act by inhibiting protein synthesis as bacteriostatic agents. After diffusion through the inner cytoplasmatic membrane, tetracyclines bind reversibly to the receptors on the 39S subunit of the ribosome, interfering with the binding of the aminoacyl-transfer RNA to the RNA-ribosome complex (Guiguère et al., 2006). Tetracyclines are broad-spectrum antibacterials, widely used for the treatment of human and animal infections. Tetracyclines are often used in slaughter pigs presenting infection following tail biting (oxytetracycline), respiratory diseases and enteric diseases caused by *Lawsonia intracellularis* (doxycycline and chlortetracycline). Tetracycline, chlortetracycline and oxytetracycline have a similar spectrum of antibacterial activity and pharmacokinetic profile. Doxycycline is a semi-synthetic tetracycline that presents a longer half-life and high lipid-solubility than other tetracyclines. Tetracyclines are rapidly but poorly absorbed, except residues which are bound to bone, and are rapidly excreted with little or no transformation via the urine and faeces. Residues of tetracycline in kidney and liver are the highest comparing to other edible tissues. Available data indicates that tetracyclines present low toxicity and there is no evidence of genotoxic or carcinogenic potential.

The marker residue for tetracycline, chlortetracycline and oxytetracycline is the sum of the parent drug and its 4-epimers. For doxycycline, the parent compound is the marker residue since no epimerization occurs. Residues of chlortetracycline are more easily detected in microbiological assays compared to tetracycline and oxytetracycline. By the use of chemical methods, residues of tetracycline and oxytetracycline are generally more easily quantified at lower levels than chlortetracycline (Food and Agriculture Organization of the United Nations and the World Health Organization, 2007).

Russian authorities require that tetracycline residues in pork are below 7.2-12.8µg/Kg. Non compliance with these limits might result in increased control and often in temporary restrictions to imports. In 2008, following the detection of doxycycline residues in Danish pork by Russian food authorities, a temporary import ban was introduced on several Danish slaughterhouses. Hence, to verify compliance with Russian requirements, in 2008, Danish abattoirs tested 591 samples using HPLC methods. Out of the 591 samples tested, 10 presented doxycycline or oxytetracycline residues, even though some of the residues were found below the HPLC quantification limit. In response to this, the industry decided to extend the withdrawal period of tetracyclines in general to 30 days.

Since 2009, the DVFA and the Danish abattoirs test, each year, 50 and 100 samples, respectively. In 2009, only 1 sample was found positive for oxytetracycline (<28µg/Kg). In 2010, LC-MS methods have also been used which allows identifying and quantifying residues at lower concentrations. In 2010, until July, out of 100 samples tested, only 1 was found positive for doxycycline (7µg/Kg), below the limit required by Russian authorities.

Macrolides

Macrolides act by inhibiting protein synthesis by reversibly binding to 50S ribosome subunit. By inhibiting the transpeptidation and translocation, they lead to premature detachment of incomplete peptide chains. Macrolides generally act as bacteriostatics but can be bactericidal at high concentrations and are active against gram-positive bacteria, *Mycoplasma* and some gram-negative bacteria (Guiguère et al., 2006). They are mainly used to treat *Lawsonia intracellularis* infections and to a lesser extent, respiratory diseases in slaughter pigs.

Tylosin is the macrolide most commonly used in pigs and is exclusively registered for veterinary use. Before the growth promoter ban, tylosin was used as the most important growth promoter in the Danish pig production. After the withdrawal, increased use of macrolides for therapeutic purposes was registered, but since 2001 it has been decreasing (National Food Institute and Statens Serum Institut, 2009). Tylosin is widely distributed in body fluids and tissues and residue depletion studies showed that tylosin residues are generally low and deplete rapidly. When administered by injection, tylosin residues are highest in the injection site and kidney; when orally administered, residue concentration is higher in liver. Metabolism mainly occurs in the liver and tylosin A is the most abundant residue and hence the marker residue identified. Tylosin presents low acute oral toxicity. Carcinogenicity studies suggesting effects on the pituitary-gonadal axis require further clarification (European Agency for the evaluation of veterinary medicinal products, 1997; Food and Agriculture Organization of the United Nations and the World Health Organization, 2007).

Tulathromycin is a semi-synthetic macrolide used for treatment of bacterial respiratory diseases in pre-weaning pigs. Pharmacokinetic studies of intramuscular administration in pigs showed rapid absorption from the injection site and long half-life especially in the lung target tissue. Oral administration was associated with lower availability. The marker residues are tulathromycin equivalents. Studies conducted indicated that tulathromycin has no carcinogenic or neurotoxic potential. Residue depletion studies indicated that residue concentration was higher in the kidney, injection sites and liver.

Pleuromutilins

Pleuromutins act by inhibiting protein synthesis binding the 50S subunit of the bacteria. Tiamulin is a strong inhibitor of peptidyl transferase (Guiguère et al., 2006).Tiamulin is a semi-synthetic derivative of pleuromutilin, exclusively used in animals and is active against gram-positive bacteria and *Mycoplasma*. Due to the synergistic effect, tiamulin is often used in combination with chlortetracycline for treatment of respiratory and enteric infections in sows and slaughter pigs. Following oral administration, tiamulin is well absorbed and extensively metabolized. Depletion studies in pigs indicate that residue concentration is higher in liver then in the kidney, muscle, skin or fat. The sum of the metabolites that may be hydrolyzed to 8-α-hydroxymutilin were identified as the marker residue in pigs. No evidences of teratogenicity or genotoxicity were found (European Agency for the evaluation of veterinary medicinal products, 1999b).

Penicillins

Penicillins inhibit the activity of transpeptidase and other penicillin-binding proteins interfering with peptidoglycan synthesis which prevents bacterial cell wall synthesis. Hence, penicillins have a bactericidal action, lysing cells undergoing cell wall synthesis (Guiguère et al., 2006).

Penicillins have been widely used in veterinary medicine for many years and are active against gram-positive bacteria. Penicillins are eliminated rapidly after absorption, mainly excreted unchanged in urine and in bile. MRLs refer to the parent compound and it is believed that, at this level, allergenic metabolites will not pose a risk to consumers. No teratogenic effects have been reported. Direct toxicity to humans and animals is minimal and non-allergic toxic effects have only been reported at very high doses and are assumed to be associated with compounds associated with penicillins in medicinal products. The 4-plate method is an appropriate method for screening; LC-MS for quantifying the marker residue (European Agency for the evaluation of veterinary medicinal products, 2008).

β-lactamase sensitive penicillins include benzylpenicillin (penicillin G) which is commonly used for treatment of Mastitis-Metritis-Agalactia Syndrome (MMA) in sows and respiratory infections in slaughter pigs. Benzylpenicillin is a combination of two compounds: benzylpenicillin and procaine. Procaine prolongs the pharmacological effect of benzylpenicillin by reducing its solubility. Following dissolution of the procaine-penicillin complex, procaine is extensively metabolized and rapidly eliminated and hence the withdrawal time required for benzylpenicillin is assumed to protect consumers from potential procaine residues (European Agency for the evaluation of veterinary medicinal products, 1998).

Amoxicillin is an extented spectrum penicillin, commonly used in combination with clavulanic acid which acts as an irreversible inhibitor of most bacteria β-lactamases. Amoxicillin is commonly used in slaughter pigs for treatment of pleuropneumonia (*Actinobacillus pleuropneumoniae).* Depletion studies showed that clavulanic acid is rapidly metabolized and the parent compound (marker residue) represents 10% of the total residues. Among the different edible tissues, the kidney presents the higher clavulanic acid residue concentrations.

Sulfonamides

Sulfonamides present a selective bacteriostatic action and act by interfering with the folic acid synthesis by avoiding para-aminobenzoic acid incorporation (Guiguère et al., 2006). Sulfonamides are commonly used in veterinary medicine as chemotherapeutics of bacterial and protozoal diseases. They are frequently administered in combination with trimethoprim which is active against *E. coli* and some *Klebsiella*, *Proteus* and *Staphylococcus* sp. for treatment of MMA and urinary tract infection in sows. Sulfonamide residue depletion varies according to different factors including the nature of the compound and the route of administration, among others. Toxicological data, carcinogenicity and mutagenicity studies are currently not available. Potential adverse effects include allergic reactions in humans. Hence, a MRL of 100 µg/Kg was applied to all compounds of the sulfonamide group (European Agency for the evaluation of veterinary medicinal products, 1995). Trimethoprim is considered to be of low acute toxicity and potentially teratogenic. Trimethoprim is usually excreted faster than sulfonamides. Hence, if no sulfonamide residues are detected, no trimethoprim residues are expected to be found (European Agency for the evaluation of veterinary medicinal products, 2002).

In 1990, following the detection of sulfadimidine residues by United States and Japan authorities, the Danish authorities decided to ban the use of sulfadimidine in sows and slaughter pigs. The ban was changed into an industry ban in 1997, since the Danish authorities were not able to maintain the ban due to EU legislation.

Lincosamides

Lincosamides act by binding to the 50S ribosomal unit inhibiting peptidyl transferases and consequently inhibiting protein synthesis. Depending on the concentration and bacterial species, lincosamides can have a bacteriostatic or bactericidal action (Guiguère et al., 2006).

Lincomycin belongs to the lincosamides group and is commonly used in mono-preparations and in combination with other antibiotics, namely spectinomycin. Lincomycin is active against Gram-positive bacteria and is commonly used in slaughter pigs for treatment of mycoplasma pneumonia (*Mycoplasma pneumoniae*) and mycoplasma arthritis (*Mycoplasma hyosynoviae*).

Studies have shown that lincomycin presents low acute toxicity and no genotoxicity.

Data from residue depletion studies show that lincomycin represents all microbiological activity of incurred residues and hence is the recommended marker residue. In pigs, highest concentrations of residues are presented in kidney and liver.

ANNEX C

THE DUTCH RESIDUE SURVEILLANCE PROGRAM

In 2004, the NAT was introduced in the Dutch residue surveillance program replacing the New Dutch Kidney Test (NDKT) (Nouws et al., 1988; Pikkemaat et al., 2008a).

The Dutch residue surveillance comprises both random and suspect sampling (Michel Rapallini, personal communication). In the random sampling scheme, carcasses are not detained independently of the screening or post-screening test result. On the other hand, in the suspect sampling scheme, in case of a positive post-screening result, carcasses are detained until chemical confirmation results are obtained. Suspect pigs are identified based on visual inspection (injection site, abnormalities that might indicate disease history), incomplete farm records and finally, previous findings (farms that tested positive in the past will be sampled more intensively).

At screening, 34 samples can be tested in the same plate. On average, the cost per sample at screening is approximately 0.5€. At post-screening, seven samples can be tested on the same plate. The post-screening cost depends on the test plate, but on average costs 1€ (Mariel Pikkemaat, personal communication).

Cost data of the screening Nouws antibiotic test

Material	Amount per plate	Cost per plate (€)
Iso-Sensitest Agar (IST) (B&M-plate)	125 ml	2.07
Iso-Sensitest Agar (IST) (T-plate)	125 ml	2.07
Plate count agar (DIFCO) (Q-plate)	125 ml	2.91
DST-agar (S-plate)	125 ml	7.70
Plate count agar (DIFCO) (A-plate)	125 ml	2.91
Positive control (B&M-plate)	1	0.40
Positive control (T-plate)	1	0.40
Positive control (Q-plate)	1	0.40
Positive control (S-plate)	1	0.40
Positive control (A-plate)	1	0.40

Cost data of the post-screening Nouws antibiotic test

Test plate	Material	Amount per plate	Cost per plate (€)
B&M-plate	Iso-Sensitest Agar (IST)	30 ml	0.72
	M. luteus	1	0.02
T-plate	Iso-Sensitest Agar (IST)	30 ml	0.72
	B. cereus	1	0.02
Q-plate	2/3 Plate count agar (DIFCO)	30 ml	0.55
	Y. ruckeri	1	0.02
S-plate	DST-agar	50 ml	2.57
	B. pumilus	1	0.02
A-plate	Plate count agar (DIFCO)	30 ml	0.70
	B. pumilus	1	0.,02

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