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Swedish Veterinary Antimicrobial
Resistance Monitoring



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Preface

WELCOME to the eleventh Swedish report combining results from the monitoring of antimicrobial resistance and antimicrobial usage in both veterinary and human medicine: SVARM and SWEDRES. These two reports are printed jointly to increase the awareness of trends in incidence of use and occurrence of antimicrobial resistance in the respective areas.

Antimicrobial resistance is a good example of the relevance of the one health concept. Fundamental is that bacteria in animals, humans and in the environment are interlinked by several direct or indirect routes. Thereby resistant bacteria can spread within and between human communities, animal populations and the environment. One of the routes connecting intestinal bacteria of animals and humans is food. It is therefore our intention to further develop the studies in SVARM on resistance in bacteria from food.

Not only the epidemiology of resistance is complex but also the actions needed to counteract resistance comprise a multitude of aspects. Paramount is reduction of the selection pressure exerted by use of antimicrobials. This is best achieved by reducing the need for antimicrobials by prevention of diseases in animals and humans. In veterinary medi-

cine, preventive herd health is a well known concept. Herd health programs combine knowledge from diverse fields such as animal husbandry, composition of animal feed and disease control. If properly designed, such programs can be instrumental to mitigate emergence and spread of resistance in animal populations.

An important aspect of strategies against resistance is reliable diagnostics, including susceptibility testing. In the clinical setting, diagnostics are needed to support the prudent and rational choice of therapy. But diagnostics is also vital for appraisal of the situation regarding resistant strains in herds of animals or in patients at veterinary clinics. Thus, it is only possible to manage what can be measured and only if the situation is known can effective action be taken. In a wider context, monitoring programs provide knowledge needed for informed decisions on interventions and also measure the effects of interventions. It is our hope and belief that the data presented in SVARM will be useful for appraisal of the situation on a national level and in a wider context also adds a piece to the global puzzle on antimicrobial resistance.



Guidance for readers

Cut-off values for resistance

In SVARM, isolates of indicator bacteria and zoonotic bacteria are classified as susceptible or resistant by epidemiological cut-off values (ECOFF) issued by EUCAST and available online at www.eucast.org. Also animal pathogens are classified by ECOFFs when such values are available. Cut-off values used are given in Appendix 4.

ECOFFs classify isolates with acquired reduced susceptibility as non-wild type, in SVARM called “resistant”. This classification is relevant for monitoring purposes, but it should be understood that this does not always imply clinical resistance.

Since the first report from SVARM, some cut-off values for resistance have been changed. To facilitate comparisons when retrospect data are presented in SVARM 2010, levels of resistance have been recalculated using current cut-off values if not otherwise stated.

Indicator bacteria

In SVARM, *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium* serve as indicators for presence of antimicrobial resistance in the enteric flora of healthy animals and in the flora contaminating retail meat. The prevalence of acquired resistance in such commensal bacteria indicates the magnitude of the selective pressure from use of antimicrobials in an animal population. Most bacteria of the enteric flora are unlikely to cause disease, but they can be reservoirs for resistance genes that can spread to bacteria that cause infections in animals or humans. Prevalence of resistance in bacteria contaminating meat indicates the magnitude of the potential risk of human exposure to such reservoirs in food producing animals.

Presentation of MIC distributions

Susceptibility data are presented as distributions of MICs in tables of a uniform design as below. Distributions are given as percentages of isolates tested. In the tables, white fields denote range of dilutions tested for each substance and vertical bold lines indicate cut-off values used to define resistance.

The percentage of isolates with a certain MIC for an antimicrobial is given in the corresponding white field. For MICs above the range tested for an antimicrobial (> XX mg/L) the percentage is given in the field closest to the range, i.e. in the first shaded field to the right of the tested range. For MICs equal to or lower than the lowest concentration tested for an antimicrobial (\leq YY mg/L) the percentage is given as the lowest tested concentration, i.e. in the first white field of the tested range.

Example of a table with distributions of MICs:

Antimicrobial	Resistance (%)	Distribution (%) of MICs (mg/L)											
		≤ 0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Ciprofloxacin	21	21.0	52.0	6.0			1.0			20.0			
Erythromycin	0				93.0	4.0	3.0						
Tetracycline	2		75.0	22.0	1.0			1.0	1.0				

Multiresistance

The term “multiresistance” is used in SVARM with a meaning as proposed by Schwarz et al. (2010). Briefly, isolates with phenotypically identified acquired resistance to three or more antimicrobial classes are deemed multiresistant. This implies for example that resistance to ciprofloxacin, enrofloxacin and nalidixic acid represents resistance to one class of antimicrobials.

Antimicrobial abbreviations

Am	ampicillin	Fu	fusidic acid
Ba	bacitracin	Gm	gentamicin
Ce	ceftiofur	Km	kanamycin
Ci	ciprofloxacin	Na	narasin
Cl	clindamycin	Nal	nalidixic acid
Cm	chloramphenicol	Ox	oxacillin
Col	colistin	Sm	streptomycin
Ct	cephalothin	Su	sulphonamide
Ctx	cefotaxime	Tc	tetracycline
Ef	enrofloxacin	Tp	trimethoprim
Em	erythromycin	Va	vancomycin.
Fox	cefoxitin	Vi	virginiamycin
Ff	florfenicol		

Other abbreviations

ESBL	extended spectrum beta-lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MIC	minimum inhibitory concentration
MRSA	methicillin resistant <i>Staphylococcus aureus</i>
MRSP	methicillin resistant <i>Staphylococcus pseudintermedius</i>
VRE	vancomycin resistant enterococci

Summary

THE 2010 REPORT FROM SVARM shows that the situation regarding antimicrobial resistance in bacteria from animals remains favourable from an international perspective. However, the importance of continuous monitoring as a tool to discover introduction of new types of resistance and to identify trends is again manifested. In SVARM 2010, occurrence of resistance to 3rd generation cephalosporins and an increase in quinolone resistance in *Escherichia coli* from broilers is reported. Also reported is the first isolation of methicillin resistant *Staphylococcus aureus* of the livestock associated type (MRSA CC398) from Swedish pigs. These examples illustrate a constantly changing situation where only informed actions on several levels, national as well as local, can counteract spread and thereby mitigate the consequences of resistance. The measurable improvement in “prudent use” of antimicrobials for companion animals is a good example of a situation where effective activities were initiated from knowledge of trends gained by monitoring.

The total amount of antimicrobials used for animals was 14 177 kg in 2010, which is the lowest figure in 30 years. The amount of antimicrobials for in-feed or in-water medication has decreased by 38% since 2006 and is today but 10% of the total sales. This reflects a marked decrease in sales of macrolides, pleuromutilins and tetracyclines for pigs. The sales of antimicrobials for dogs have decreased by 22% since 2006 measured as total number of prescriptions dispensed. Prominent decreases in sales of cephalosporins, aminopenicillins with clavulanic acid and fluoroquinolones are noted. The downward trend in prescriptions for dogs is explained by ongoing national and local work with improved implementation of hygiene and prescribing policies.

Salmonella is rare in Swedish animals and most incidents involve susceptible strains. In 2010, 87% of the strains were susceptible to all antimicrobials tested and only 6 of 62 strains from food producing animals and none of 20 strains from companion animals and wildlife were multiresistant. Resistance to 3rd generation cephalosporins was not observed. There are no indications of increased occurrence of resistance but in view of the public health consequences vigilance towards resistant *Salmonella* in food-producing animals is warranted. This is emphasised by the occurrence in later years of multiresistant monophasic *Salmonella* subspecies I, O 4,5,12;i- in animals.

In broilers *Campylobacter jejuni* resistant to quinolones (21%) was more common in 2010 than in previous years. The reasons for this are not known. Selection through use of antimicrobials is unlikely since quinolones are seldom used in broiler production in Sweden. Continuous monitoring to follow up the finding is needed as well as further studies to elucidate the epidemiology of quinolone resistant *Campylobacter*.

Methicillin resistant *Staphylococcus aureus* (MRSA) in animals is notifiable to the Board of Agriculture. In 2010, MRSA was confirmed in four dogs, two cats, five horses and in one sample from pigs. Since first reported in 2006 and until the end of 2010, MRSA have been isolated from 18 cases in dogs, 4 in cats, 15 in horses and in one sample from pigs. The isolate from pig, the first from food producing animals in Sweden, was of spa-type t011 and belonged to the livestock associated CC398. Most isolates from dogs and cats were of spa-types that are common among MRSA from humans in Sweden. In contrast, all but two isolates from horses are of spa-type t011 associated to MRSA CC398. This type is common in several animal species in other countries but rare among humans in Sweden. Since there is a zoonotic aspect to MRSA in animals the situation should be closely monitored. Also, routines and recommendations for prevention of spread, as well as for management of MRSA in animals, should be elaborated.

Resistance in indicator bacteria (*Escherichia coli* and *Enterococcus* spp.) from the intestinal flora of healthy animals, are believed to reflect the antimicrobial selective pressure in an animal population. Also, indicator bacteria from food reflect the exposure of humans to resistant bacteria from food animals. Resistance in indicator bacteria from broilers was low and reflected by an equally low level in indicator bacteria on broiler meat. However, resistance to quinolones in *E. coli* from broilers has increased from 2% in 2002 to 13% in 2010. Moreover, screening by more sensitive selective cultures revealed *E. coli* with cephalosporinases of extended spectrum beta-lactamase (ESBL)- or AmpC-type in 34% of samples from broilers. These findings are puzzling and cannot be explained by antimicrobial use in Swedish broiler production. Preliminary findings indicate introduction and spread from imported breeding stock.

In this year's report indicator bacteria from horses are presented for the first time. In *E. coli*, the dominating findings were resistance to streptomycin, sulphonamides or trimethoprim (13-16%). Multiresistance occurred in 12% of the isolates. Resistance to 3rd generation cephalosporins was not found. However, on screening faecal samples by selective culture SHV producing *E. coli* was isolated from six of 431 samples. In *Enterococcus faecalis* a quadruple resistance; macrolides, aminoglycosides (gentamicin and kanamycin), tetracyclines and chloramphenicol was found in six isolates (18%). Resistance was rare in *Enterococcus faecium*.

Vancomycin resistant enterococci (VRE) were isolated from 23% of 200 samples of intestinal content from broilers and from 2 of 100 samples of broiler meat. Samples were cultured on vancomycin supplemented media. Prevalence of VRE in Swedish broilers increased to a peak of 41% in 2005 and has since declined. This year, the prevalence is about the same as in 2006-2009 indicating a stable situation.

Isolates of *Escherichia coli* from clinical submissions from pigs and calves were often resistant to ampicillin, streptomycin, tetracycline or trimethoprim-sulphonamides. In *E. coli* from horses resistance to streptomycin and trimethoprim-sulphonamides were the most common traits whereas in isolates from dogs and cats resistance to ampicillin dominated. Multiresistance varied, ranging from 3% in isolates from cats to 38% in isolates from calves.

Since 2008, production of ESBL has been confirmed in 28 isolates of *Enterobacteriaceae* from dogs, cats and horses. Beta-lactamases involved were of groups CTX-M-1 or SHV and all the isolates were multiresistant.

In isolates of *Brachyspira* spp. from pigs, resistance to tiamulin occurred in *B. pilosicoli* but was not observed in *B. hyodysenteriae*. The majority of isolates of *B. pilosicoli* and *B. hyodysenteriae* were resistant to tylosin.

Resistance was rare in isolates of *Actinobacillus pleuropneumoniae* and *Pasteurella* spp. from the respiratory tract of pigs as well as in *Pasteurella* spp. from the respiratory tract of calves. However, penicillin resistance in *Mannheimia haemolytica* from calves was confirmed in one herd.

In *Aeromonas salmonicida* subsp. *achromogenes*, *Flavobacter columnare* and *Flavobacter psychrophilum* from farmed fish, deviating high MICs to nalidixic acid, tetracycline or florfenicol

in some isolates indicate acquired resistance to these antimicrobials.

Isolates of *Streptococcus zooepidemicus* from the respiratory tract of horses were uniformly susceptible to penicillin, but resistance to trimethoprim-sulphonamides occurred.

Beta-lactamase production was the most common resistance trait in *Staphylococcus aureus* from skin samples of horses (21%) but only less than 1% were multiresistant. One isolate was methicillin resistant and confirmed as MRSA.

Most isolates of *Staphylococcus pseudintermedius* from the skin of dogs were resistant to penicillin through beta-lactamase production. Resistance to clindamycin, erythromycin, fusidic acid or tetracycline was also common (20-31%). One third of the isolates of *S. pseudintermedius* were multiresistant and 8% were resistant to at least five antimicrobials. In addition, 4% were resistant to oxacillin and a majority of these were confirmed methicillin resistant. In Sweden isolates of methicillin resistant *S. pseudintermedius* (MRSP) are notifiable. During 2010, 100 isolates from dogs and five from cats in Sweden were reported to the Board of Agriculture.

Isolates of *Pseudomonas aeruginosa* from the external ear canal of dogs were susceptible to polymyxin B, whereas 2% of the isolates were resistant to gentamicin and 20% to enrofloxacin.



Sammanfattning

SVARM 2010 visar att resistensläget hos bakterier från djur är fortsatt gynnsamt ur ett internationellt perspektiv. Men rapporten visar också återigen på vikten av regelbunden övervakning för att upptäcka introduktion av nya resistenstyper. I SVARM 2010 rapporteras till exempel resistens mot 3^e generationens cefalosporiner och en ökad förekomst av kinolonresistens hos *Escherichia coli* från slaktkyckling. Dessutom rapporteras det första fallet hos svenska grisar av meticillinresistent *Staphylococcus aureus* av den typ (MRSA CC398) som är vanlig bland vissa av lantbrukets djur i många andra Europeiska länder. Exemplet illustrerar en ständigt föränderlig situation där bara kunskapsbaserade åtgärder på flera nivåer, lokal såväl som nationell och internationell, kan motverka spridning och följderna av antibiotikaresistens. Ett gott exempel där kunskap från övervakning av resistens och förskrivning omsatts i verkningfulla åtgärder är den mätbara ökningen av "ansvarsfull" antibiotikaanvändning till sällskapsdjur som dokumenterats i SVARM.

Försäljningen av antibiotika till djur var totalt 14 177 kg under 2010, vilket är den lägsta siffran på 30 år. Volymen antibiotika för inblandning i foder eller vatten har minskat med 38 % sedan 2006 och utgör idag endast 10 % av den totala försäljningen. Förändringen beror på en kraftig minskning av försäljning av makrolider, pleuromutiliner och tetracykliner för gris. Antalet recept som skrivs ut för hund har minskat med 22 % sedan 2006. Förskrivningen av cefalosporiner, aminopenicilliner med klavulansyra och fluorokinoloner har minskat kraftigt. Den nedåtgående trenden avseende försäljning för hund är resultatet av intensivt arbete med hygien och antibiotikapolitik nationellt och lokalt.

Fynd av **meticillinresistent *Staphylococcus aureus* (MRSA)** hos djur är anmälningspliktiga till Jordbruksverket. Under 2010 påvisades MRSA hos fyra hundar, två katter, fem hästar och i ett prov från grisar. Det positiva provet från gris var det första från livsmedelsproducerande djur i Sverige. Sedan det första fallet hos svenska djur 2006 har MRSA konfirmerats hos 18 hundar, 4 katter, 15 hästar och i ett prov från grisar till och med 2010. De flesta isolat från hundar och katter är av spa-typer som är vanliga bland MRSA från människor i Sverige. De flesta isolat från hästar tillhör spa-typ t011 liksom isolatet från gris. Spa-typ t011 är ovanlig i svensk sjukvård men vanlig bland MRSA (CC398) från livsmedelsproducerande djur i många länder. MRSA betraktas som ett zoonotiskt smittämne och läget i djurpopulationer bör därför övervakas. Dessutom bör det utarbetas rutiner och rekommendationer för hur spridning av MRSA kan motverkas liksom rekommendationer för hantering av djur med MRSA.

Salmonella är ovanligt hos svenska djur och de fall som inträffar orsakas oftast av antibiotikakänsliga stammar. Under 2010 var 87 % av isolaten känsliga för alla testade antibiotika. Bara 6 av 62 isolat från livsmedelsproducerande djur och inget av 20 isolat från sällskapsdjur och vilda djur var multiresistenta. Inget isolat var resistent mot 3^e generationens cefalosporiner. Inget tyder på en ökad förekomst av resistens men situationen är ändå föränderlig. Detta illustreras av att det nu också hos svenska djur finns multiresistent monofasisk *Salmonella* subspecies I, O 4,5,12;i- som sedan några år sprids hos människor och djur i flera länder. Sedan 2006 har 7 utbrott bland svenska lantbruksdjur med denna salmonellatyp dokumenterats.

Resistens mot kinoloner bland *Campylobacter jejuni* (21 %) från slaktkyckling var vanligare under 2010 än tidigare år. Orsaken är inte känd men troligen beror ökningen inte på selektion genom antibiotikaanvändning eftersom kinoloner sällan används till slaktkyckling i Sverige. Epidemiologiska förhållanden kommer att klarläggas genom ytterligare undersökningar och utvecklingen kommer att följas genom fortsatt övervakning.

Resistens hos indikatorbakterier (*Escherichia coli* och *Enterococcus* spp.) ur tarmfloran hos friska djur anses återspegla selektion av resistens pga. av användning av antibiotika till djuren. Indikatorbakterier från livsmedel ger en uppfattning om resistent bakterier från lantbruksdjur som kan nå människor via livsmedelskedjan.

Hos slaktkyckling var resistens generellt ovanlig hos indikatorbakterier från såväl tarminnehåll som från kycklingkött. Men kinolonresistens i tarminnehåll har ökat från 2 % 2002 till 13 % 2010. Med känsligare selektiv odlingsmetod påvisades *E. coli* med överförbar resistens mot 3^e generationens cefalosporiner (extended spectrum beta-lactamases, ESBL, - eller AmpC) i 34 % av prov av tarminnehåll. Varken ökad kinolonresistens eller förekomst av resistens mot cefalosporiner kan förklaras med användning av dessa antibiotika till svensk slaktkyckling. Preliminära resultat visar att orsaken istället är spridning av resistent bakterier från importerade avelsdjur.

I årets SVARM presenteras för första gången resistens hos indikatorbakterier från häst. Hos *E. coli* från träckprov dominerade resistens mot streptomycin, sulfonamider eller trimetoprim (13-16 %). Tolv procent av isolaten var multiresistenta. Resistens mot tredje generationens cefalosporiner påvisades inte men när träckprov odlades på selektiva media påvisades SHV-producerande *E. coli* i 6 av 431 prov. Hos *Enterococcus faecalis* var kvadrupel resistens mest framträdande och sex isolat (18 %) var resistent mot makrolider, aminoglykosider (gentamicin och kanamycin), tetracykliner och kloramfenikol. Resistens hos *Enterococcus faecium* var ovanligt.

Vankomycinresistenta enterokocker (VRE) i tarminnehåll från slaktkyckling påvisades under 2010 i 23 % av 200 prov från tarminnehåll och i 2 av 100 prov av kycklingkött. Resultaten baseras på undersökningarna med s.k. selektiv metod med hög känslighet. Andelen positiva prov är densamma som 2006-2009 och lägre än 2005 då det var 41%, vilket visar att läget nu är stabilt.

Isolat av *E. coli* från kliniska prov från grisar och kalvar var ofta resistenta mot ampicillin, streptomycin, tetracyklin eller trimetoprim-sulfa. Hos isolat av *E. coli* från hästar var resistens mot streptomycin och trimetoprim-sulfa vanligast medan resistens mot ampicillin dominerade bland isolat från hundar och katter. Frekvensen multiresistens varierade mellan djurslag och var lägst (3 %) hos isolat från katter och högst (38 %) hos isolat från kalvar.

Sedan 2008 har produktion av ESBL ur grupperna CTX-M-1 eller SHV konfirmerats hos 28 isolat av *Enterobacteriaceae* från hundar, katter och hästar. Alla isolaten var multiresistenta. Hos isolat av *Brachyspira pilosicoli* från grisar förekom resistens mot tiamulin men däremot inte bland isolat av *B. hyodysenteriae*. Majoriteten av såväl *B. pilosicoli* som *B. hyodysenteriae* var resistenta mot tylosin.

Isolat av *Actinobacillus pleuropneumoniae* och *Pasteurella spp.* från luftvägarna hos grisar liksom *Pasteurella spp.* från kalvar med luftvägssjukdom var känsliga för de flesta antibiotika som används för behandling. Under 2010 påvisades dock resistens mot penicillin hos *Mannheimia haemolytica* isolerad från kalvar i en besättning.

Bland *Aeromonas salmonicida* subsp. *achromogenes*, *Flavobacter columnare* och *Flavobacter psychrophilum* från odlad fisk förekom isolat med avvikande höga MIC-värden mot nalidixansyra, tetracyklin eller florfenikol. Detta tyder på att vissa isolat förvärvat resistens mot dessa antibiotika.

Isolat av *Streptococcus zooepidemicus* från luftvägarna hos hästar var genomgående känsliga för penicillin men resistens mot trimetoprim-sulfa förekom.

En stor andel isolat av *Staphylococcus aureus* från huden på hästar var resistenta mot penicillin genom produktion av betalaktamas (21 %) men mindre än 1 % var multiresistenta. Ett isolat var meticillinresistent och konfirmerades som MRSA.

Isolat av *Staphylococcus pseudintermedius* isolerade från hudprover från hundar var i stor utsträckning resistenta mot penicillin. Resistens mot klindamycin, erytromycin, fusidinsyra, eller tetracyklin var också vanligt (20-31 %). En knapp tredjedel av isolaten var multiresistenta och 8 % var resistenta mot minst fem antibiotika. Resistens mot oxacillin (4 %) var vanligare än tidigare och de flesta av dessa isolat var meticillinresistenta. Fynd av **meticillinresistent S. pseudintermedius (MRSP)** är anmälningspliktiga och enligt uppgift från Jordbruksverket anmäldes MRSP hos 100 hundar och fem katter under 2010.

Isolat av *Pseudomonas aeruginosa* från yttre hörselgången hos hund var alla känsliga för polymyxin B, medan 2 % av isolaten var resistenta mot gentamicin och 20 % mot enrofloxacin.



Use of antimicrobials

STATISTICS ON TOTAL SALES of antimicrobials for use in animals in Sweden are available since 1980. For a review of the data from 1980-2000 as well as references to publications on which that review is based, see SVARM 2000. Data represent an approximation of the real use of antimicrobials, assuming that the amount sold is also used during the observation period. Data for 2010 were provided by Apotekens Service AB. Details on source of data and inclusion criteria are given in Appendix 2 and on antimicrobial agents with general marketing authorisation in Sweden in Appendix 4.

Trends in animal populations

Changes in the numbers of animals may affect trends in statistics on use of antimicrobials. The number of beef cows have increased by 4% in five years (i.e. since 2006), but the number of dairy cows has decreased by 10%. The number of pigs slaughtered has been roughly unchanged in the last five years (-3%), while the number of broilers was 9% higher in 2010 than in 2006. The number of horses was 363 000 in 2010, an estimated increase by 10-20% since 2004. Details on animal numbers are found in Appendix 1.

Overall use

The total yearly sales of antimicrobials over the last decade are presented in Table AC I. The potency of different antimicrobials is not equal and therefore each class should be evaluated separately. Nevertheless, the overall figures may indicate general trends. In the following, trends over the last five years (i.e. since 2006) will be commented. The total sales expressed as kg active substance have decreased by 18 % in five years. The sum for 2010 is the lowest hitherto reported (for earlier data see SVARM 2000). Pronounced decreases (more than 20%) are noted for all classes of antimicrobials except for penicillin and aminopenicillins. Long term trends in total sales of classes that are currently marketed are illustrated in Figure AC I a & b. Comments on recent trends are given in relation to animal species (see below).

Of the total sales, 90% are products formulated for treatment of individual animals (injectables, tablets, intramammaries) and 10% for treatment of groups or flocks (premixes, oral powders, solutions for water medication). In table AC II, the sales of products for use in individual animals, excluding topical, intrauterine and intramammary use are presented. The sales of cephalosporins (almost entirely first generation cephalosporins) have decreased by 53% in five years, almost entirely related to decreased prescription of first generation cephalosporins for dogs. The sales of fluoroquinolones for therapy of individual animals have decreased by 24% since 2006. This is explained both by a marked decrease in sales of fluoroquinolones for oral use in dogs and cats (34% decrease of that subset) and of products for injection (20% decrease of that subset).

Data on sales of antimicrobials formulated for medication of groups of animals are given in Table AC III. Data for 1984 are given for historical reference. In Figure AC II, the development of sales of veterinary medicines formulated for medication of groups of animals and of antimicrobial feed additives (before 1986) is shown. Substances grouped as 'others' are the feed additives and other substances that are no longer available on the market (e.g. nitroimidazoles). Overall, the sales of products intended for administration to groups of animals have decreased by 96% since 1984. This long term reduction is not only explained by the cessation of growth promoting use, as the decrease since 1990 is 91%. In the last five years, the sales of veterinary medicines for group medication have decreased by 38%. Pronounced decreases are noted for all groups except fluoroquinolones, where the use has been at or below 5 kg during the five year's period, and aminopenicillins which have come into use for pigs and poultry on special license prescription.

Information on the repartition of the volumes sold of the different classes to 'food producing animals including horses' and 'companion animals' has been taken from the latest available report from the Swedish Board of Agriculture on sales of veterinary medicinal products (Table AC IV). A large proportion of the aminopenicillins and cephalosporins are used for companion animals. Also, macrolides & lincosamides and fluoroquinolones are to a considerable extent sold for use in dogs and cats. The current system does not permit full repartition of the antimicrobials sold to specific animal species.

Comments on trends by animal species

In the following, an attempt is made to comment on some trends in the use of various classes for different animal species. The discussion is based on information from different sources, e.g. species when given on the prescriptions, knowledge on how different products are generally used in Sweden and on other available information. The comments have varying degrees of uncertainty, depending on the source of information used.

Dairy cows

The Swedish Dairy Association publishes a yearly report related to the organization's work to improve animal health and welfare in dairy cows (Swedish Dairy Association, 2010). The reporting year is from September to August which in the following will be given as, e.g., 2009/10. The report includes statistics on disease incidence in dairy cows enrolled in the Swedish milk recording scheme. Data are mainly retrieved from a database with veterinary reported disease events and treatments (Jansson Mörk, 2010).

The incidence of systemic treatments with antimicrobials for all conditions in dairy cows was 24.2 and 21.6 treatments per 100 cow-years in 2001/02 and 2009/10, respectively.

Treatment with penicillin was by far the most common, and incidence of such treatment has decreased from 18.1 to 16.8 treatments per 100 cow-years in 2001/02 and 2009/10, respectively (-7%). This decrease is reflected in the statistics on sales of products for use in individual animals as benzylpenicillin has decreased by 10% since 2001 (Table AC II). The incidence of treatment with fluoroquinolones and cephalosporins has varied between 2.2 and 2.5 and 0.4 and 0.8 treatments per 100 cow-years, respectively.

tetracyclines, macrolides and pleuromutilins have decreased by 36, 49 and 63% since 2006. Trends in sales of tetracyclines are affected by changing relative proportions of doxycycline versus other tetracyclines within the class. Doxycycline has a higher bioavailability and the dose is lower (250 ppm when mixed in feed) compared with e.g. chlortetracycline (1000 ppm when mixed in feed). Dose and population corrected figures on sales of tetracyclines indicate that use of this class has decreased by 53% since year 2007 (Figure AC III).

TABLE AC I. Yearly sales of antimicrobial drugs for veterinary use expressed as kg active substance. Based on sales statistics provided by Apoteket AB and from Apotekens Service AB.

ATCvet code	Antimicrobial class	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
QJ01AA, QG01A	Tetracyclines ^a	1 453	1 415	1 307	1 329	1 562	1 516	1 853	1 649	1 174	1 115
QJ01CE, -R, QJ51	Benzylpenicillin ^b	8 414	8 179	7 579	7 814	7 571	7 860	7 582	7 758	7 721	7 546
QJ01CA, QJ01CR	Aminopenicillins	752	767	870	875	911	920	927	938	1 068	907
QJ01D	Cephalosporins	474	676	832	928	1 009	1 217	954	820	738	575
QA07AA, QJ01G, -R, QJ51R	Aminoglycosides and polymyxins ^a	770	753	645	606	762	750	718	643	609	557
QA07AB, QJ01E	Sulphonamides	2 485	2 477	2 326	2 462	2 535	2 543	2 427	2 303	2 128	1 998
QJ01E	Trimethoprim & derivatives	414	414	381	406	437	450	438	416	379	357
QJ01F	Macrolides & lincosamides	1 510	1 412	1 124	1 095	1 080	1 254	1 520	1 096	988	739
QJ01MA	Fluoroquinolones	182	185	184	187	184	195	180	169	164	148
QJ01XX92, -94	Pleuromutilins	841	988	744	387	338	459	506	572	398	174
Total		17 295	17 266	15 992	16 089	16 389	17 164	17 106	16 364	15 368	14 117

^a Includes drugs marketed with special licence prescription for years 2000-2006; ^b Also includes small amounts of penicillinase stable penicillins.

The most common indication for antimicrobial treatment of Swedish dairy cows is mastitis. In 2009/10, the number of reported events of clinical mastitis was 14.2 per 100 cow-years. In Sweden, mastitis is generally treated systemically and any changes in treatment incidence, treatment length or choice of antimicrobial for this condition will have a noticeable influence on the statistics on sales of antimicrobials. When intramammarys are used during lactation, it is usually to supplement the systemic treatment. The sales of products for intramammary use during lactation has decreased from 398 dose applicators/1000 cows in 2006 to 357 dose applicators/1000 cows in 2010. Almost all (99%) of the sales of intramammarys for use during lactation were products with penicillin or penicillin combined with aminoglycosides. The sales of products for intramammary use at drying off has been roughly unchanged and was 672 dose applicators/ 1000 cows in 2010. Assuming that 4 dose applicators are used per cow, this corresponds to 17 treatments per 100 cow-years. Products with penicillin combined with aminoglycosides are the most commonly used for prevention around drying off (98% of the sales).

Pigs

Antimicrobials for group treatment are mainly used for pigs except for aminopenicillins which are also used for poultry, and for fluoroquinolones which are mainly used for poultry but also for other species. The sales of all other antimicrobial classes in Table AC III reflect use for pigs. The sales of

The drop in use of macrolides and tetracyclines is likely to reflect improved knowledge on how to manage problems with concomitant infections in herds with postweaning multisystemic wasting syndrome. This includes the introduction of vaccination strategies and an awareness that in most cases, antimicrobials have no or limited effect.

The main indication for pleuromutilins (tiamulin, valnemulin) is swine dysentery. It is probable that efforts to control the disease through e.g. a certification programme resulted in a decreased need to treat swine dysentery, leading to overall declining sales figures since the mid 90s (Figure AC I).

Under certain conditions the farmer may administer antimicrobials to pigs for specified diseases under supervision of the farm veterinarian (as specified in a regulation from the Swedish Board of Agriculture, SJVFS 2009:84). In such cases, the farmer keeps detailed records of all treatments, and the veterinarian visits the farm regularly. This practice is likely to be applied on all pig farms. The sales of injectable antimicrobials for pigs (i.e. the amounts sold from pharmacies with pig given as species on the prescription) are likely to be a good approximation of the use of antimicrobials for individual pigs. The sales of benzylpenicillin for pigs have increased by 32% over the last five years. In 2010, 59% of the sales of antimicrobials for use individual pigs were penicillin, and an additional 5% were penicillin combined with aminoglycosides. Trimethoprim combined with sulphonamides was the second largest class sold (22%) and the sales have increased by 17%

over the last five years. A shift from medication of groups of animals via feed or water towards medication of individual clinically diseased animals, preferably with narrow spectrum antibiotics such as penicillin, is well in line with the rational use of antimicrobials.

Poultry

Antimicrobials are rarely used for treatment of bacterial diseases in commercially reared *Gallus gallus* (broilers, laying hens or parent birds). Localized outbreaks at broiler farms or problems in a breeding facility can therefore have a major influence on the sales in a specific year. In 2010, the total sales where *Gallus gallus* was given as species was 44.5 kg of which 78% were aminopenicillins. The indication for treatment with aminopenicillins is occasional outbreaks of diseases. Over the last five years, the yearly sales of fluoroquinolones for *Gallus gallus* have been below or much below 1.5 kg. The total sales for turkeys in 2010 amounted to 33 kg of which 96% were aminopenicillins and most of the remainder were fluoroquinolones.

Coccidiostats of the ionophore group are used as feed additives to control coccidiosis in the production of chickens for slaughter and for turkeys. Since the late 80s, narasin is by far the most widely applied substance.

Horses

The sales of trimethoprim and sulphonamides have increased steadily until 2006 but since, there has been a decrease by 20% (table AC II). In the last five years, around two thirds of the sales in this category were products for oral use in horses (paste or powder). Thus, the trend largely reflects use in horses. As noted under 'Trends in animal numbers', the number of horses has increased by 10-20% since 2004. Using that estimate to correct for the increasing population, the number of doses of paste or powder (pasta tubes or dose powders) per 1000 horses has decreased by 23-25% since 2006.

The sales of other antimicrobials for horses is difficult to estimate, as they are frequently administered by the veterinar-

ian in connection with an examination, either in ambulatory practice or in clinics or hospitals.

Dogs

The total sales of antimicrobials for oral use in dogs expressed as total number of prescriptions has decreased by 22% since 2006 (Figure AC IV). The dataset includes prescriptions for drugs authorised for systemic oral use in animals (ATC vet code QJ01) as well as for humans (ATC code J01) and corresponds to out-patient use for dogs. The most recent estimates of the dog population in Sweden are from 2006, but there are no indications that the number of animals has decreased.

The most prominent changes relative to 2006 was noted for cephalosporins (-51%), aminopenicillins with clavulanic acid (-33%), fluorquinolones (-32%) and lincosamides (+15%). In 2010, aminopenicillins was the largest class sold (36% of the total sales in prescriptions), followed by lincosamides (20%) and the proportion of first generation cephalosporins, amoxicillin with clavulanic acid and fluoroquinolones 13, 12 and 11%, respectively. Figures on sales of antimicrobials for dogs expressed as kg active substance have only been calculated for the products authorised for use in animals. The sales of that subset represent around 95% of the total sales for dogs. Based on prescription statistics, the total sales of veterinary antimicrobials for oral use in dogs was 1337 kg in 2010, representing about 10% of the total sales of veterinary antimicrobials in Sweden. Trends per substance class are similar to those discussed for the prescriptions (see also 'overall use'). The trend since 2006 in sales of antimicrobials for dogs expressed as kg active substance is more pronounced (-37%) than the corresponding decrease in number of prescriptions or packages (figure AC IV).

As described in SVARM 2008, the emergence of infections with multiresistant methicillin resistant *Staphylococcus pseudintermedius* and methicillin resistant *S. aureus* triggered a number of national and local initiatives. This has most likely led to changes in prescribers' behaviour, which in turn explains the downward trends in sales of antimicrobials for dogs.

TABLE AC II. Yearly sales of antimicrobial drugs authorised for individual treatment expressed in kg active substance. Only products for systemic use (QJ01) or for use as intestinal anti-infective (QA07) are included. Based on sales statistics from Apotekens Service AB.

ATCvet code	Antimicrobial class	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
QA07A	Intestinal anti-infectives ^a	614	594	594	586	496	434	372	364	355	302
QJ01A	Tetracyclines	623	628	606	611	623	609	632	605	576	538
QJ01CE	Benzylpenicillin ^{b, c}	8 343	8 127	7 536	7 769	7 493	7 777	7 504	7 671	7 641	7 492
QJ01CA-CR	Aminopenicillins	752	767	870	875	911	909	899	828	802	742
QJ01D	Cephalosporins	474	676	832	928	1 009	1 212	950	817	735	575
QJ01E	Sulfonamides & trimethoprim	2 478	2 483	2 280	2 427	2 610	2 689	2 619	2 486	2 270	2 138
QJ01F	Macrolides & lincosamides	522	477	430	382	400	417	413	352	332	311
QJ01G	Aminoglycosides ^c	454	460	367	344	362	345	343	318	301	274
QJ01M	Fluoroquinolones	169	178	177	180	179	190	177	164	159	144
QJ01X	Pleuromutilins	48	49	77	32	29	39	36	36	28	17
Total		14 477	14 439	13 769	14 134	14 112	14 622	13 944	13 640	13 198	12 532

^a Drugs marketed with special licence prescription are included from year 2000; includes aminoglycosides, formolsulfatiazole and colistin; ^b The amount includes QJ01R; ^c Does not include the aminoglycosides in QA07A, intestinal anti-infectives.

TABLE AC III. Yearly sales of antimicrobial drugs authorised for group treatment and ionophoric anticoccidials sold expressed as kg active substance. Based on sales statistics from Apotekens Service AB and from the Board of Agriculture

ATCvet code	Antimicrobial class	1984	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
QA07A	Intestinal anti-infectives ^a		-	-	-	-	163	170	158	106	107	119
QJ01A	Tetracyclines	12 300	822	777	695	712	934	903	1 217	1 040	594	575
QJ01C	Penicillins incl. aminopenicillins	-	-	-	-	-	-	11	28	111	266	164
QJ01F	Macrolides & lincosamides	607	988	935	694	713	680	837	1 107	744	657	427
QJ01MA	Fluoroquinolones	-	13	7	8	7	5	5	3	5	5	4
QJ01MQ	Quinoxalines ^b	9 900	-	-	-	-	-	-	-	-	-	-
QJ01XX91	Streptogramins ^c	8 800	-	-	-	-	-	-	-	-	-	-
QJ01XX92, -94	Pleuromutilins	-	793	939	667	355	309	420	471	536	370	157
QP51AA	Nitroimidazoles	1 440	-	-	-	-	-	-	-	-	-	-
	Feed additives ^d	700	-	-	-	-	-	-	-	-	-	-
Total		33 747	2 616	2 658	2 064	1 787	2 091	2 346	2 984	2 543	1 999	1 447
QP51AH	Ionophoric antibiotics (coccidiostats) ^{d, e}	7 900	10 019	8 439	10 920	10 486	11 095	12 335	12 527	13 376	12 471	NA ^e

^a Drugs with special licence prescription are included from year 2005, includes aminoglycosides and colistin; ^b Years 1980-1984 sold as feed additives, thereafter on veterinary prescription at therapeutic dosages until 1997; ^c Feed additives other than quinoxalines and streptogramins: avoparcin, bacitracin, nitrovin, oleandomycin and spiramycin; ^d Figures are from the Feed Control of the Board of Agriculture (www.sjv.se); ^e Not available at the time of publication.

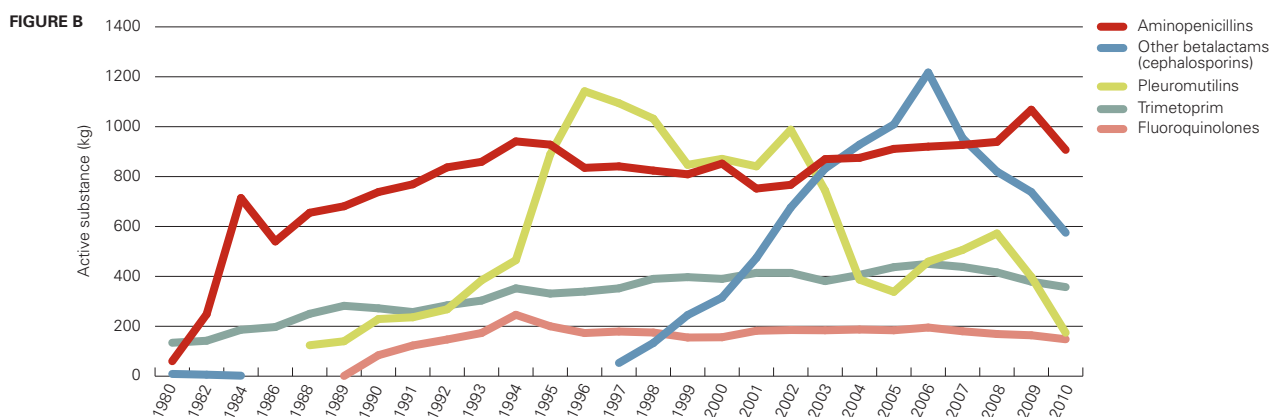
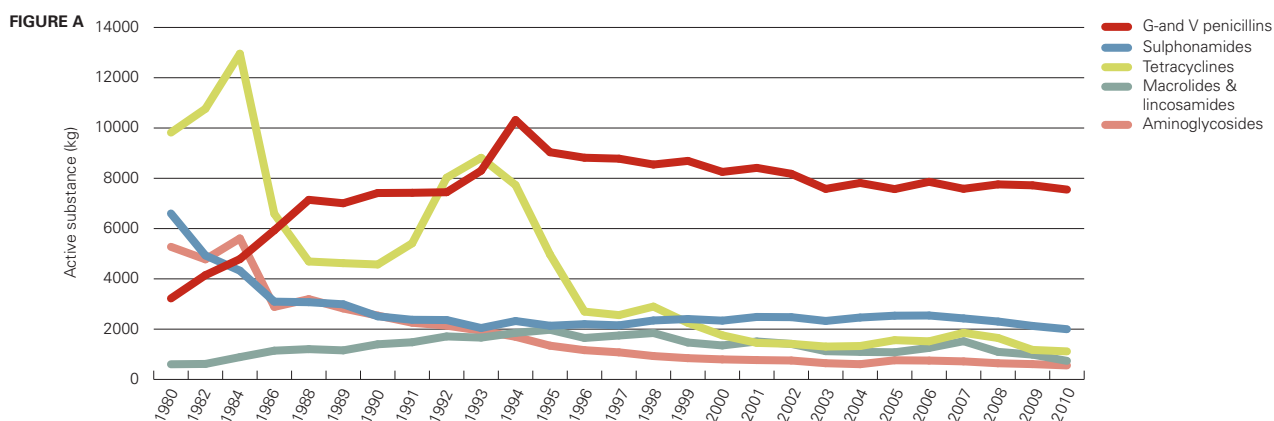


FIGURE AC I A & B. Sales of antimicrobials for animals. Amphenicols, nitroimidazoles, streptogramins, quinoxalines and other feed additives were withdrawn from the market during the time period and are not shown. Note that the scales on the Y-axis are different in figure a and b. Based on sales statistics provided by Apoteket AB and by Apotekens Service AB.

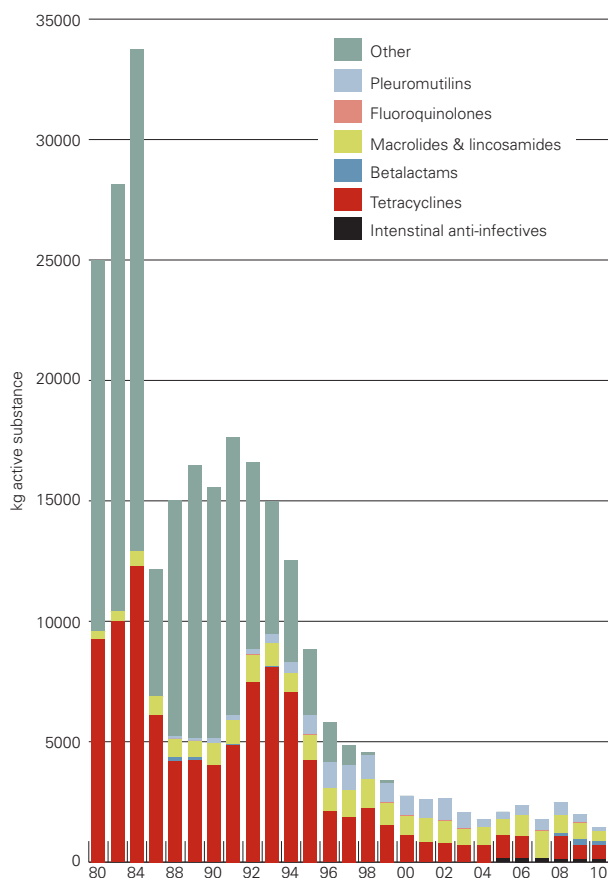


FIGURE AC II. Yearly sales of antimicrobial drugs authorised for group treatment measured as kg active substance (based on Table AC III and data from SVARM 2000).

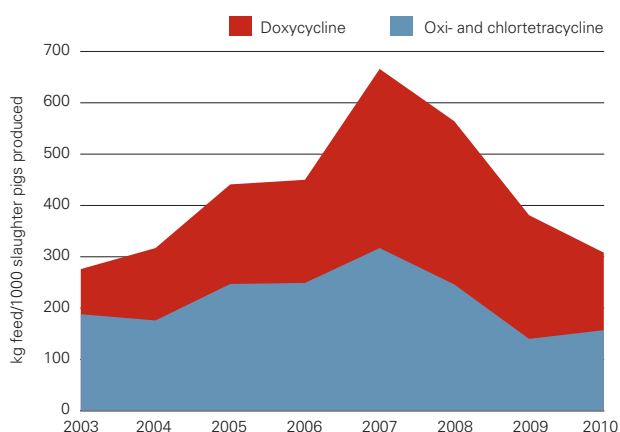


FIGURE AC III. Yearly sales of tetracyclines for group treatment calculated as kg feed per 1000 pigs slaughtered.

TABLE AC IV. Sales of antimicrobial drugs in 2009 (kg active substance) per category of animals.

Antimicrobial class ^a	Food producing animals (incl horses)	Companion animals	Other or unknown
Tetracyclines	90%	8%	2%
Penicillin G & V	95%	4%	1%
Aminopenicillins	28%	63%	8%
Cephalosporins	3%	97%	0%
Aminoglycosides & polymyxins	81%	16%	2%
Sulphonamides & trimethoprim	89%	11%	<1%
Macrolides & lincosamides	69%	23%	8%
Fluoroquinolones	70%	30%	<1%
Pleuromutilins	100%	0%	0%

Data are from the Swedish Board of Agriculture's report on usage of veterinary medicines (www.jordbruksverket.se; in Swedish), based on sales statistics provided by Apotekens Service AB.

^a For included ATC groups see table AC I.

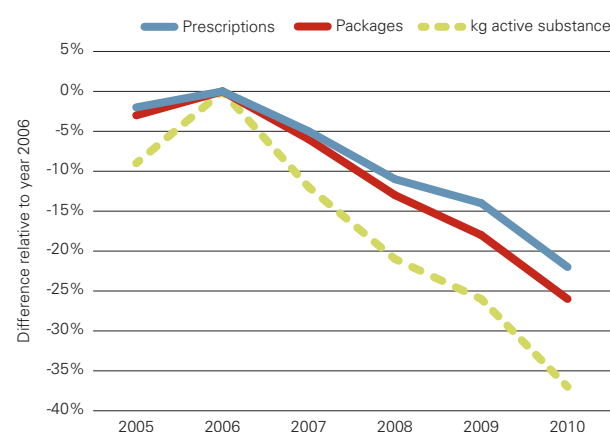


FIGURE AC IV. Sales of antimicrobials for systemic use in dogs. packages and prescriptions are for all antimicrobials (QJ01 and J01) and kg active substance of drugs only authorised for veterinary use (QJ01). Based on sales data from Apotekens Service AB.

Zoonotic bacteria

ZOONOSES ARE DISEASES and infections that can be naturally transmitted between animals and man. Antimicrobial resistance in zoonotic bacteria such as *Salmonella*, *Campylobacter* and methicillin resistant *Staphylococcus aureus* (MRSA) from animals is therefore of direct public health concern. Here data regarding these bacteria in Swedish animals are presented. More information on infections with zoonotic bacteria in Sweden is presented in the yearly report *Surveillance of zoonotic and other animal disease agents in Sweden*, available at www.sva.se.

Salmonella

Findings of *Salmonella* in animals are notifiable in Sweden and antimicrobial susceptibility is tested in one isolate from each warm-blooded animal species (wild and domesticated) involved in an incident. In incidents involving more than one serotype or phage type, one isolate of each serotype and phage type was tested. In SVARM 2010, isolates from incidents notified in 2010 are included but also isolates from incidents notified previously but still under restrictions. In addition, isolates obtained in the salmonella surveillance programme from samples collected at slaughter are included. For details on methodology see Appendix 3.

Results and comments

The overall situation with regard to *Salmonella* among Swedish animals is favourable from an international perspective. Occurrence of *Salmonella* among food-producing animals is low, most likely as a result of the strategies in the Swedish Salmonella control programme, and few incidents involve multiresistant strains.

All animals 2010

Altogether 82 isolates were tested of which about half were *S. Typhimurium* and four were of the monophasic serotype O 4,5,12:i:- (Table Salm I). The majority of all isolates (87%) were susceptible to all antimicrobials tested but 11 isolates were resistant to at least one substance (Table Salm II).

The resistant isolates were from nine separate incidents of which eight involved food producing animals (Table Salm II). One incident in cattle and one in horses involved *S. Typhimurium* resistant to sulphonamides and trimethoprim. The incident in horses was revealed already in 2009. The incident in cattle occurred in the same geographical area as the incident in horses but epidemiological links were not established.

One incident in cattle involved *S. Typhimurium* DT 120 resistant to ampicillin, streptomycin, sulphonamide and tetracycline. In another incident in cattle *S. Typhimurium* NST resistant to streptomycin, sulphonamide and tetracycline was

isolated. The latter phenotype is previously not confirmed in *S. Typhimurium* from Swedish animals (Table Salm VII).

In three incidents, monophasic *Salmonella enterica*, (subspecies I, O 4,5,12:i:-) was isolated. Two of these incidents involved cattle and were epidemiologically linked. The third incident involved both cattle and poultry. Isolates from all three incidents were resistant to ampicillin, streptomycin, sulphonamide and tetracycline.

Finally, one incident in pigs and another in cattle involved salmonella resistant to streptomycin at low MICs (32 mg/mL) and one incident in pigs involved isolates resistant to streptomycin and sulphonamide.

Food-producing animals 2000-2010

From a public health perspective resistance in *Salmonella* from food-producing animals is more important than resistance in isolates from wild animals or pets. In the period 2000-10, 506 isolates from notified incidents in food-producing animals have been tested in SVARM. This includes isolates from the vast majority of notified incidents in food-producing animals in the period.

Of these isolates, 238 (47%) were *S. Typhimurium*. Most of these isolates (41%) were from pigs, 28% were from cattle, 29% from poultry and 2% from sheep. Distributions of MICs and occurrence of resistance among these isolates are given in Table Salm VI. Fifty-five (23%) of isolates of *S. Typhimurium* were resistant to at least one antimicrobial and 17 isolates (7%) to three or more substances, i.e. they were multiresistant (Table Salm VII). Among serovars other than *Typhimurium*, 10 isolates (4%) were multiresistant.

The 17 multiresistant isolates of *S. Typhimurium* are from 16 separate incidents of which 11 involved only cattle, two involved pigs only and one incident involved both pigs and cattle. Of the remaining incidents one was in sheep and one in ducks in a hobby flock. Three incidents in 2004 involving cattle were epidemiologically linked through trade of calves. An epidemiological link is also suspected between four incidents 2007-2008 involving cattle, pigs and sheep. Links between the other incidents are unknown. Resistance phenotypes of the isolates are given in Table Salm VI.

Monophasic *Salmonella* subspecies I, O 4,5,12:i:- was first confirmed in Swedish animals in 2006. In all, seven incidents involving this serotype have been disclosed in food-producing animals. Four incidents involved cattle, one incident each in swine or ducks and one incident involving both cattle and poultry. In all incidents isolates have been multiresistant with the same phenotype; ampicillin-tetracycline-streptomycin-sulphonamide. Epidemiological links have been confirmed in some of the incidents.

TABLE SALM I. Number of *Salmonella enterica* tested for antimicrobial susceptibility, 2010.

Serotype	Cattle	Pig	Sheep	Poultry	Ostrich	Horse	Dog	Cat	Wild		Total
									birds	mammals	
S. Agona	1										1
S. Derby		2									2
S. Dublin	5										5
S. Enteritidis, not phage typed	1								1		2
S. Enteritidis, NST		1									1
S. Enteritidis, 4		1									1
S. Livingstone				2							2
S.Oranienburg		1									1
S. Reading	4	1				1	1	1	1		9
S.Saintpaul	1										1
S. Senftenberg					1						1
S. Teshie	1										1
S. Thompson		1									1
S. Typhimurium, not phage typed	2	2		1		1		4	2		12
S. Typhimurium, DT 120	2			1							3
S. Typhimurium, NST	3	2		10				1			16
S. Typhimurium, DT 1	1							1			2
S. Typhimurium, NST, U277		1						2			3
S. Typhimurium, DT 39	1										1
S. Typhimurium, DT 41	1					1			1		3
S. Typhimurium, NT				1							1
S. Typhimurium, DT 125	1										1
S. Typhimurium, DT 146	1										1
S. enterica subsp. diarizonae (IIIb)	1		2								3
S. enterica (I) O 4,5,12:i:-	3			1							4
S. enterica (III) O 50:i:-		1									1
S. enterica (I) O 4,5:i:-1,5										1	1
S. enterica O 6,8:i:-										1	1
S. enterica (I) O 4:i:-		1									1
Total	29	14	2	16	1	3	1	9	5	2	82
Percent of total	35%	17%	2%	20%	1%	4%	1%	11%	6%	2%	

TABLE SALM II. MICs (mg/L) of *Salmonella enterica* resistant to at least one antimicrobial, 2010. Shaded fields indicate resistance.

Animal species	Sevovar	Am	Ctx	Cm	Ff	Gm	Km	Ci	Nal	Sm	Su	Tc	Tp
Horse	S. Typhimurium, not phagetyped	1	≤0.06	4	4	1	2	0.03	4	8	>1024	1	>32
Cattle	S. Typhimurium DT146	1	≤0.06	4	4	1	2	0.03	4	16	>1024	1	>32
Cattle	S. Typhimurium DT 120	>64	0.12	4	4	1	2	0.03	8	256	>1024	>64	≤0.25
Cattle	S. Typhimurium NST	1	0.12	4	4	1	2	0.03	8	>256	>1024	>64	0.25
Cattle	S. enterica (I) O 4,5,12:i:-	>64	0.12	4	4	0.5	2	0.03	4	256	>1024	>64	≤0.25
Cattle	S. enterica (I) O 4,5,12:i:-	>64	≤0.06	4	4	1	4	0.03	4	>256	>1024	>64	≤0.25
Cattle	S. enterica (I) O 4,5,12:i:-	>64	0.12	4	4	0.5	2	0.06	8	>256	>1024	>64	≤0.25
Poultry	S. enterica (I) O 4,5,12:i:-	>64	0.12	4	4	1	2	0.06	8	256	>1024	>64	≤0.25
Pig	S. enterica (I) O 4:i:-	1	0.25	8	8	1	4	0.06	8	256	>1024	2	0.5
Pig	S. enterica (III) O 50:i:-	≤0.5	≤0.06	≤2	≤2	0.5	2	0.016	≤2	32	64	1	≤0.5
Cattle	S. Saintpaul	1	≤0.06	4	4	1	2	0.03	4	32	128	1	1

TABLE SALM III. Percent resistance (R) and distribution of MICs for *Salmonella enterica* (n=82) 2010.

Antimicrobial	R (%)	Distribution (%) of MICs (mg/L)																		
		≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Ampicillin	6						9.8	79.3	4.9							6.1				
Cefotaxime	0			58.5	40.2	1.2														
Chloramphenicol	0								24.4	74.4	1.2									
Ciprofloxacin	0	1.2	84.1	14.6																
Florfenicol	0							19.5	78.0	2.4										
Gentamicin	0				2.4	42.7	53.7	1.2												
Kanamycin	0					1.2	2.4	61.0	35.4											
Nalidixic acid	0							1.2	82.9	15.9										
Streptomycin	11							1.2	4.9	17.1	65.9	2.4				4.9		3.7		
Sulphonamide	11											1.2	6.1	56.1	25.6					11.0
Tetracycline	7							72.0	20.7							7.3				
Trimethoprim	2					68.3	28.0	1.2								2.4				

TABLE SALM IV. Percent resistance (R) and distribution of MICs for *Salmonella* Typhimurium (n=43) 2010.

Antimicrobial	R (%)	Distribution (%) of MICs (mg/L)																		
		≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Ampicillin	2						9.3	83.7	4.7							2.3				
Cefotaxime	0			62.8	37.2															
Chloramphenicol	0								27.9	72.1										
Ciprofloxacin	0		93.0	7.0																
Florfenicol	0							27.9	72.1											
Gentamicin	0					27.9	69.8	2.3												
Kanamycin	0							44.2	55.8											
Nalidixic acid	0								90.7	9.3										
Streptomycin	5									11.6	83.7						2.3	2.3		
Sulphonamide	9											7.0	65.1	18.6					9.3	
Tetracycline	5							79.1	16.3							4.7				
Trimethoprim	5					72.1	23.3									4.7				

TABLE SALM V. Percent resistance and source of isolates for *Salmonella* Typhimurium 1978-2010, all animals.

Antimicrobial	Cut-off value (mg/L)	Resistance (%)								
		1978-88 ^a (n=125)	1989-99 (n=317)	2000-02 (n=108)	2003-05 (n=183)	2006 (n=52)	2007 (n=71)	2008 (n=63)	2009 (n=67)	2010 (n=43)
Ampicillin	>8	2	6	3	7	15	7	11	3	2
Cefotaxime	>0.5	-	-	-	0	0	0	0	0	0
Ceftiofur	>2	-	-	0	0	0	-	-	-	-
Chloramphenicol	>16	4 ^b	5 ^b	3	6	2	1	8	3	0
Ciprofloxacin	>0.06	-	-	-	-	0	0	3	1	0
Enrofloxacin	>0.25	-	1	0	<1	-	-	-	-	-
Florfenicol	>16	-	-	3	4	2	1	8	3	0
Gentamicin	>2	-	0 ^b	6	<1	0	0	0	0	0
Kanamycin	>16	-	-	-	-	0	0	0	0	0
Nalidixic acid	>16	-	-	4	<1	0	0	2	1	0
Neomycin	>4	0 ^b	1 ^b	4	0	-	-	-	-	-
Streptomycin	>16	74	15	35	19	13	3	29	7	5
Sulphonamide	>256	-	-	3	7	13	6	11	7	9
Tetracycline	>8	13	6	3	7	10	3	10	3	5
Trimethoprim	>2	-	-	0	<1	0	0	0	4	5
Trim/sulph.	>0.5/9.5	0	3	-	-	-	-	-	-	-
Percent of isolates from:										
Cattle, sheep, pigs, poultry		100	46	45	21	40	53	70	49	70
Horses, cats, dogs			29	36	65	36	17	16	31	23
Wildlife			25	19	14	24	30	14	19	7

^a 1988 includes isolates to September, isolates from October-December 1988 given under 1989; ^b Cut-off value for resistance >8 mg/L.

TABLE SALM VI. Percent resistance (R) and distribution of MICs for *Salmonella* Typhimurium (n=238) 2000-2010, food-producing animals.

Antimicrobial	R (%)	Distribution (%) of MICs (mg/L)																		
		≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Ampicillin	9							3.8	71.0	15.1	1.3				8.8					
Cefotaxime ^a	0			30.0	63.5	6.5														
Ceftiofur ^b	0							29.0	68.0	3.0										
Chloramphenicol	5									11.8	80.3	2.9				0.8	4.2			
Ciprofloxacin ^c	<1			61.8	37.6			0.6												
Enrofloxacin ^d	0			54.3	42.0	3.7														
Florfenicol	5										92.4	2.5	0.4		4.6					
Gentamicin	2							16.4	71.8	10.1		1.7								
Kanamycin ^e	0									29.3	66.9	3.2	0.6							
Nalidixic acid	<1									1.3	78.2	14.7	5.0		0.4				0.4	
Streptomycin	20										0.4	18.1	61.8	11.8	2.5	2.1	2.1	1.3		
Sulphonamide	10														52.0	32.4	5.9			9.6
Tetracycline	7								37.4	50.0	5.5		1.7	0.8	2.1	2.5				
Trimethoprim	<1							38.7	55	6.3					0.4					

^a 170 isolates tested; ^b 100 isolates tested; ^c 157 isolates tested; ^d 81 isolates tested.

TABLE SALM VII. Resistance phenotypes (%) of *Salmonella* Typhimurium (n=238) from food-producing animals, 2000-2010. All isolates were tested for susceptibility to ampicillin, ceftiofur/cefotaxime, enrofloxacin/ciprofloxacin, florfenicol, gentamicin, chloramphenicol, nalidixic acid, streptomycin, sulphamethoxazole, tetracycline, and trimethoprim.

Resistance phenotype	Animal species	Phage type																	Not typed	Total					
		1	7	9	10	12	15A	39	40	41	99	104	120	125	126	146	193	195			U277	NT	NST		
AmFfCmNalSmSuTcCi	Pig										1													1	
AmFfCmSmSuTc	Cattle										5	1											1	7	
AmFfCmSmSuTc	Pig										1												1	2	
AmFfCmSmSuTc	Sheep										1													1	
AmCmSmSuTc	Cattle										1													1	
AmSmSuTc	Cattle											1									2			3	
AmSmSuTc	Poultry																				1			1	
SmSuTc	Cattle																					1		1	
AmSu	Cattle											2												2	
AmSu	Pig											1												1	
GmSm	Cattle									1														1	
GmSm	Pig								1															1	
GmSm	Poultry									1														1	
SmSu	Poultry						2																	2	
SuTp	Cattle															1								1	
Am	Poultry																					2		2	
Gm	Poultry																					1		1	
Nal	Pig				1																			1	
Sm	Poultry									1												3		4	
Sm	Pig								4	3		1	1								1	4	1	15	
Sm	Cattle										1	1		1								3		6	
Susceptible	Cattle	4			2		1	1	1	4			5	1	1						1	18	6	45	
Susceptible	Pig	1	1			2			33	5	1	1	5								1	2	16	8	76
Susceptible	Sheep	1																						3	4
Susceptible	Poultry	1		1		1				4	1			2				1	1	1	4	39	2	58	
Number of isolates		7	1	1	2	4	3	1	43	16	1	15	16	1	2	1	1	1	2	11	87	22	238		
percent of total		3	<1	<1	<1	2	1	<1	18	7	<1	6	7	<1	<1	<1	<1	<1	<1	<1	5	37	9		

Campylobacter

The isolates of *Campylobacter jejuni* tested are from caecal content of broilers collected at abattoirs and were isolated within the framework of the Swedish *Campylobacter* control programme 2010. For details on methodology see Appendix 3.

Results and comments

Of the 100 isolates tested, 75 were susceptible to all six antimicrobials. Resistance to quinolones only (ciprofloxacin and nalidixic acid) was the most common phenotype (Table Camp I).

Quinolone resistance in *C. jejuni* is usually caused by a mutation in the *gyrA* gene (Bachoual et al. 2001). The mutation usually leads to high MICs for both ciprofloxacin and nalidixic

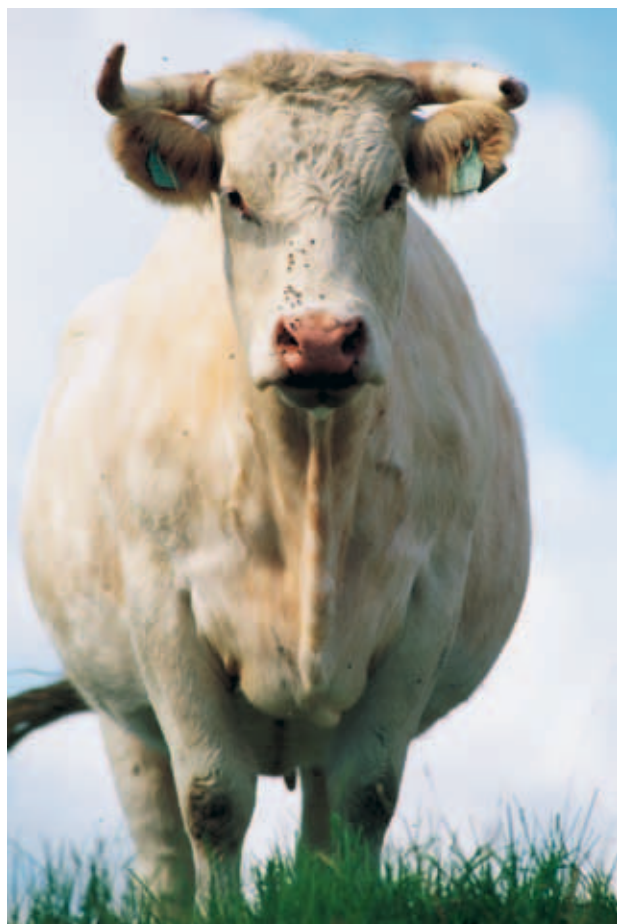
acid, the most common phenotype in this study. However, in rare instances the same mutation causes high MICs to ciprofloxacin and only moderately elevated MICs to nalidixic acid (Bachoual et al., 2001; Said et al., 2010). Notably three isolates in the present study where of this phenotype, i.e. resistant to ciprofloxacin (MICs >8 mg/L) and susceptible to nalidixic acid (MICs 4–8 mg/L).

In comparison to previous years quinolone resistance has increased notably (Table Camp I). The reasons for this are not known. But selection through use of antimicrobials is unlikely as single explanation since quinolones are seldom used in broiler production in Sweden. Further monitoring to follow up the finding is needed as well as further studies to elucidate the epidemiology of quinolone resistant *C. jejuni*.

TABLE CAMP I. Distribution of MICs and resistance (%) of *Campylobacter jejuni* from broilers, 2010. Previous data from SVARM are given for comparison.

Antimicrobial	Resistance (%)					Distribution (%) of MICs (mg/L)											
	2001 (n=91)	2002 (n=100)	2004 (n=97)	2008 (n=38)	2010 (n=100)	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Ciprofloxacin	2 ^a	0 ^a	5 ^a	0	21	21.0	52.0	6.0			1.0			20.0			
Erythromycin	0	0	0	0	0				93.0	4.0	3.0						
Gentamicin	-	-	1	0	1			17.0	74.0	8.0	1.0						
Nalidixic acid	5	0	5	0	18							26.0	54.0	2.0			18.0
Streptomycin	-	-	-	0	4				7.0	70.0	19.0	1.0	1.0				2.0
Tetracycline	0	1	3	0	2		75.0	22.0	1.0			1.0	1.0				

^aEnrofloxacin tested.



Methicillin resistant *Staphylococcus aureus* (MRSA)

Methicillin resistant *Staphylococcus aureus* (MRSA) is a serious global problem in human healthcare and recently MRSA has emerged in several animal species worldwide. This is of concern mainly from a public health perspective, since MRSA can be transferred between animal and man. However, MRSA cause infections in animals too and is therefore of clinical importance also in animal healthcare.

The public health significance of MRSA in animals and food was recently assessed by the Panel on Biological Hazards of the European Food Safety Authority (EFSA) (EFSA, 2009). One conclusion of the panel is that livestock-associated lineages, i.e. clonal complex (CC)398, can be a major contributor to the overall MRSA burden in countries with a low prevalence of human MRSA infections but is of less significance in countries where human infections are more common. The livestock-associated MRSA CC398 is most commonly found in pigs and other food-producing animals, but also occurs in other animals including horses and is reported from humans (EFSA 2009, CVMP 2009).

In Sweden, MRSA of spa-types correlating to CC398 (i.e. t011, t108, t034 and t571) and negative for pvl-gene (coding for Panton Valentine Leukocidin toxin) was documented in 22 humans in 2006–2010 (SWEDRES 2010). Of these, seven isolates were of spa-type t011 which is the dominating type among MRSA from pigs in Europe and also the most common spa-type among isolates from Swedish horses. Two human cases with spa-type t011 were reported in 2010 and both of them had, directly or indirectly, had contact with horses or other domestic animals (SWEDRES 2010).

In Sweden, MRSA in animals was first verified in 2006 and was made notifiable to the Swedish Board of Agriculture in 2008. Up to and including 2010 a total of 38 cases have been confirmed by the National Veterinary Institute (SVA). The current situation is summarized below.

Dogs and cats

MRSA has been confirmed in 18 dogs and 4 cats in Sweden (Table MRSA). The first Swedish animal isolate of MRSA came from a dog in 2006. Altogether seven different animal healthcare settings in different counties have been involved. Most isolates are from wound infections, mainly post operative wounds.

Eighteen of the isolates were of spa-type t032, two of t002, one of t127 and one of t020 (Table MRSA). Sixteen of the isolates were tested for presence of the pvl-gene and were negative. Most spa-types found in Swedish dogs and cats are common among MRSA from humans in Sweden. Spa-types t032 and t002 were the most common types among human isolates of MRSA in 2007 and 2008, respectively. Spa-types t032, t002 and t127 are all present among the ten most common spa-types in humans in 2010 (SWEDRES 2010). This supports the view that humans often are a source of MRSA in small companion animals (EFSA 2009, CVMP 2009).

Horses

All together, MRSA has been isolated from 15 horses in Sweden. The first isolation was made in a screening study 2007 using selective culture of nasal swabs. MRSA was isolated from one of 300 horses screened. The isolate, of spa-type t011 and CC398, was resistant to beta-lactams, gentamicin, kanamycin, tetracycline and trimethoprim (Table MRSA). During 2010, a new screening study was performed. On admission to five different animal hospitals, nasal samples were taken from randomly selected horses. In total, 284 horses were sampled and none of them was positive for MRSA.

The first documented outbreak of MRSA infections in Swedish horses occurred in 2008. At an equine hospital, six horses with postoperative wound infections were confirmed with MRSA. On screening of contact horses outside the hospital, one horse, without signs of infection, was revealed as carrier of MRSA in the nostrils. The index case of the outbreak was not established. Since then, MRSA has been confirmed in samples from two additional horses sampled at the equine hospital where the outbreak occurred, and in five horses sampled at, or after visit at, another equine hospital. Of the 15 isolates, 13 belonged to spa-type t011, while two, related to the second equine hospital, belonged to spa-type t064. All isolates shared the same susceptibility pattern and were negative for the pvl-gene.

Food-producing animals

MRSA in food-producing animals is reported globally, mostly in pigs but the prevalence is high also among veal calves and broilers and MRSA also occurs among dairy cows (for a review see EFSA, 2009). In production animals, the livestock associated MRSA CC398 dominates.

In the summer of 2010, MRSA was isolated from food-producing animals in Sweden for the first time. In a screening study, slaughter pigs from 191 herds were sampled at 6 different slaughter houses. From each herd five pigs were sampled by nasal swabs directly after slaughter and swabs from the same herd were pooled at the laboratory. One of the samples was positive for MRSA. Since the study was anonymous, the location of the positive herd is not known. The isolate belonged to spa-type t011 and CC398.

Screening studies for MRSA in pigs have been performed twice previously in Sweden. During 2006–2007, slaughter pigs on 100 pig production holdings were screened by culture of nasal swabs. None of the samples were positive. In 2008, Member States of the European Union screened holdings with breeding pigs for MRSA by culture of dust using harmonized methodology (Decision 208/55/EC). Overall MRSA was confirmed on 27% of the holdings in the EU but from none of the 208 Swedish holdings sampled.

Screening studies for MRSA in milk from Swedish dairy cattle have been performed in 2002–2003 and 2005 without positive findings. During 2010, 206 isolates of beta-lactamase producing *S. aureus* isolated from milk samples were investigated for methicillin resistance without positive findings.

In 2002–2007, MRSA was not found when coagulase positive staphylococci isolated from carcasses of broilers, pigs and cattle were tested for methicillin resistance by the National Food Administration (see Highlight “Coagulase positive staphylococci from broiler, pig and cattle carcasses”).

Future strategies

Reported incidents of MRSA in animals are still few in Sweden but the situation can rapidly change. During 2010, MRSA was detected in the pig population and the extent of the spread is not known. Sweden is still a country with a comparatively low prevalence of human MRSA infection (SWEDRES 2010) and therefore measures should be taken to avert a situation where animals constitute a reservoir for MRSA spreading into human healthcare.

As discussed in two recent reports from EU authorities, a measure to mitigate MRSA could be improved biosecurity to hinder spread to, between and within farms with food-producing animals (EFSA 2009, CVMP 2009). Other options discussed are improved infection control in animal healthcare, to prevent spread and nosocomial infections in companion

animals, and a reduction of antimicrobial selection pressure in animal populations by prudent use of antimicrobials. The reports acknowledge that basic hygiene measures such as hand washing and disinfection is of key importance to control transfer of MRSA between humans and animals. Also, it is recognised that periodical monitoring of MRSA in food producing animals is essential for decisions on control strategies and evaluation of their effect.

During 2011, active monitoring of MRSA in dairy cows continues and monitoring among breeding pigs is considered. In addition, since MRSA is notifiable there is a passive surveillance by culture of routine samples from animal healthcare. But to implement the measures with a potential to contain spread among animals, awareness of the problem among stakeholders such as farmers, animal owners, veterinarians and laboratory personnel is crucial. Awareness is likely to increase vigilance for MRSA-infections as well as compliance to recommendations on infection control and prudent use of antimicrobials. Continuous communication about relevant information and recommendations on practical measures are therefore important components of strategies against MRSA.



TABLE. Cases of methicillin resistant *Staphylococcus aureus* (MRSA) in Swedish animals up to and including 2010. All isolates were positive for the *mecA* and *nuc* genes by molecular methods. Shaded areas indicate MICs above EUCAST ECOFFs.

Animal species	Year	Clinical background	Antimicrobial													Spa-type
			Ox ^a	Fox	Pc	Ct	Cl	Em	Tc	Fu	Gm	Km	Ci	Tp	Cm	
Dog	2006	post-op wound	>16	>16	>4	8	≤0.25	0.5	≤0.5	0.5	≤0.5	2	>4	1	8	t032
Dog	2006	post-op wound	>16	>16	>4	8	≤0.25	0.5	≤0.5	0.5	≤0.5	2	>4	1	8	t032
Dog	2006	post-op wound	>16	8	>4	>8	≤0.25	0.5	≤0.5	0.25	1	4	>4	2	8	t032
Dog	2007	post-op wound	>16	>16	>4	>8	≤0.25	0.5	≤0.5	0.5	≤0.5	4	>4	2	8	t032
Dog	2007	abscess	>16	>16	>4	>8	≤0.25	0.5	≤0.5	0.5	≤0.5	2	>4	1	8	t032
Dog	2007	post-op wound	>16	>16	>4	>8	0.5	0.5	2	-	1	2	>4	2	4	t032
Dog	2007	post-op wound	>16	16	>4	8	≤0.25	0.5	≤0.5	0.25	≤0.5	2	>4	1	8	t032
Dog	2007	unknown	>16	16	>4	>8	≤0.25	0.5	≤0.5	0.25	≤0.5	4	>4	2	8	t032
Dog	2008	wound	>16	>16	>4	>8	≤0.25	1	≤0.5	0.25	1	2	>4	2	8	t032
Dog	2008	unknown	>16	>16	>4	>8	≤0.25	≤0.25	≤0.5	0.5	1	2	>4	1	8	t032
Dog	2008	unknown	>16	>16	>4	>8	≤0.25	1	≤0.5	0.25	1	2	>4	2	8	t032
Dog	2008	unknown	>16	>16	>4	>8	0.5	>32	≤0.5	0.5	32	>32	>4	>32	16	t127
Dog	2009	post-op wound	8	>16	>4	>8	≤0.25	0.5	≤0.5	0.25	≤0.5	2	>4	2	8	t032
Dog	2009	wound	>16	>16	>4	>8	0.5	1	1	0.5	1	4	>4	4	16	t032
Dog	2010	wound	>16	>16	>4	>8	>32	>32	≤0.5	0.5	1	>32	>4	2	16	t002
Dog	2010	ear	8	-	>4	>8	≤0.25	0.5	≤0.5	0.5	≤0.5	2	>4	1	8	t032
Dog	2010	unknown	>16	16	>4	8	≤0.25	>32	≤0.5	0.5	≤0.5	2	>4	8	4	t020
Dog	2010	skin	16	16	>4	1	≤0.25	<0.25	≤0.5	8	1	2	0.5	2	8	t002
Cat	2009	urine	>16	>16	>4	>8	≤0.25	0.5	≤0.5	0.25	≤0.5	0.5	>4	4	4	t032
Cat	2009	unknown	>16	>16	>4	>8	≤0.25	0.5	≤0.5	0.5	1	1	>4	2	8	t032
Cat	2010	ear	>16	-	>4	>8	≤0.25	0.5	≤0.5	1	≤0.5	2	>4	1	8	t032
Cat	2010	nose	>16	16	>4	>8	≤0.25	<0.25	≤0.5	0.25	≤0.5	1	>4	1	8	t032
Horse	2007	screening	>16	-	>4	1	≤0.25	0.5	64	0.5	>64	>32	1	>32	8	t011
Horse	2008	post-op wound	>16	>16	>4	1	≤0.25	0.5	32	0.5	64	>32	1	>32	8	t011
Horse	2008	post-op wound	>16	>16	>4	2	≤0.25	1	32	1	>64	>32	1	>32	8	t011
Horse	2008	post-op wound	16	>16	>4	2	≤0.25	1	32	0.5	>64	>32	0.5	>32	8	t011
Horse	2008	post-op wound	>16	>16	>4	2	≤0.25	0.5	32	0.25	>64	>32	0.5	>32	8	t011
Horse	2008	screening	>16	16	>4	2	≤0.25	1	32	0.5	64	>32	0.5	>32	8	t011
Horse	2008	post-op wound	>16	8	>4	2	≤0.25	1	64	1	>64	>32	1	>32	16	t011
Horse	2008	post-op wound	2	>16	4	4	≤0.25	≤0.25	32	0.12	4	32	0.25	>32	4	t011
Horse	2009	wound	16	>16	>4	>8	≤0.25	0.5	64	0.25	16	>32	0.25	>32	8	t011
Horse	2009	post-op wound	16	>16	4	1	≤0.25	0.5	32	0.25	64	>32	1	>32	8	t011
Horse	2010	post-op wound	>16	>16	>4	8	0.5	2	64	1	>64	>32	1	>32	16	t011
Horse	2010	post-op wound	>16	>16	>4	4	<0.25	1	32	0.5	>64	>32	0.5	>32	8	t064
Horse	2010	wound	>16	>16	>4	8	≤0.25	0.5	64	0.25	64	>32	0.25	>32	8	t011
Horse	2010	skin	>16	>16	>4	4	≤0.25	0.5	32	0.5	>64	>32	0.25	>32	8	t011
Horse	2010	post-op wound	>16	>16	>4	2	≤0.25	1	32	0.5	16	>32	0.25	>32	8	t064
Pig	2010	nose	>16	>16	>4	>8	0.5	1	64	0.5	>64	>32	0.25	>32	16	t011

^a tested with 2 % NaCl.

Escherichia coli with ESBL - or transferrable AmpC-type resistance in broilers

IN RECENT YEARS *Enterobacteriaceae* producing extended spectrum beta-lactamases (ESBL) has emerged as a major challenge and concern in human healthcare (Pitout & Laupland, 2008). In the last decade, occurrence in animals of *Salmonella* spp. and *Escherichia coli* with transferable genes conferring production of extended spectrum beta-lactamases or AmpC has been increasingly reported in production animals and on meat (Anonymous, 2009; Smet et al., 2010). These findings may indicate a potential for zoonotic transmission of such resistance along the food chain. (Dutil et al., 2010). The extent to which such transmission occurs is unclear, as is the magnitude of its impact on human healthcare (Smet et al., 2010). Still, reservoirs of bacteria producing extended spectrum beta-lactamases in food animals should be avoided.

In most monitoring programs in the EU, including the Swedish monitoring program SVARM, data on prevalence of resistance in *E. coli*, are based on data from randomly selected colonies from non-selective cultures. In addition, in SVARM, healthy food animals are since 2008 screened for ESBL or AmpC producing *E. coli* by culture of intestinal content on media supplemented with cefotaxime. Pigs and fattened calves were screened with negative outcome in 2008 and 2009, respectively (SVARM 2008 & 2009). In 2010, broilers were screened and ESBL or AmpC producing *E. coli* were found in a large proportion of the samples.

Methodology

In all, 200 samples of caecal content from broilers were collected at seven abattoirs processing over 99% of broilers in Sweden. Half of the samples were collected in the spring and half in the autumn 2010. Each sample represents a unique batch of broilers, but not always a unique production site.

Laboratory methods used are described in Appendix 3, SVARM 2010. Briefly, samples were cultured on MacConkey agar with cefotaxime 1mg/L. From plates with growth, isolates of *E. coli* were selected for testing of susceptibility to different antimicrobials, including cefotaxime, by microdilution according to CLSI (2010). From each sample one isolate with MIC to cefotaxime >0.25 mg/L was tested for genotype either by microarray (Anjum et al., 2007) or by multiplex PCR (Perez-Perez & Hanson, 2002; Woodford et al., 2006). In addition, the specific gene variants were determined for the isolates from the spring sampling by sequencing using in-house primers and Big-Dye™ v1.1.

Results and Comments

From 68 of the 200 samples cultured, *E. coli* with transferable cefotaxime resistance were isolated. Twelve isolates were of the CTX-M-1 genotype and 56 of the CMY-2 genotype (Table H1). In 10 isolates of the CTX-M-1 genotype sequenced, the *bla*_{CTX-M-1} was identified. Likewise, in all 22 isolates of the

CMY-2 type sequenced the *bla*_{CMY-2} gene was identified. The 68 isolates could be grouped in 11 different phenotypes based on antibiogram (Table H1 II).

Most data on prevalence of *E. coli* resistant to 3rd generation cephalosporins presented in monitoring programs, including the summary report from EFSA (EFSA 2008), are based on data from non-selective cultures. In spite of this, *E. coli* resistant to 3rd generation cephalosporins are documented in several countries in recent years (EFSA 2008). Also in Swedish broilers have *E. coli* with such resistance been documented previously since three of 296 indicator *E. coli* from the monitoring program 2007 in retrospect were confirmed to be of the CMY-2 genotype (SVARM 2007). In 2010, two isolates of CTX-M-1 genotype were found among 181 randomly selected *E. coli* isolates from intestinal content from broilers. The lower prevalence of ESBL or AmpC resistance among the randomly selected isolates indicates a low proportion of *E. coli* with such resistance in the intestinal flora. This illustrates that monitoring based on testing using non-selective cultures can underestimate the prevalence of ESBL or AmpC resistance in an animal population.

Prevalence of *E. coli* resistant to 3rd generation cephalosporins has in later years increased notably in some countries (Persoons et al., 2010a; MARAN 2008) and the increase has been linked to use of 3rd generation cephalosporins in broilers (Persoons et al., 2010b; Dutil et al., 2010). In Sweden cephalosporins are not used in broilers and thus, occurrence of *E. coli* of CTX-M-1 and CMY-2 genotypes is not associated with a selection pressure through use of such antimicrobials.

Instead a transmission of resistant bacteria from breeding stock was suspected. This was supported by findings of *E. coli* carrying genes coding for CMY-2 or CTX-M-1 in environmental samples from hatcheries hatching production animals or breeding-stock (parent animals) of both hybrids produced in Sweden collected in May to June 2010. Moreover, *E. coli* of CMY-2 genotype was found in intestinal content of day-old chickens imported as breeding stock (grand parents) in July to December 2010 and sampled on arrival to Sweden.

These findings indicate a spread of resistant *E. coli* from imported breeding stock into Swedish broiler production. The temporal variation in proportion of CMY-2 or CTX-M-1 genotypes among Swedish broilers might reflect dissemination of different lineages of resistant bacteria from different batches of imported breeding stock (Table H1 I). A similar spread along the production pyramid has been suggested for cephalosporin resistant *E. coli* (Persoons et al., 2010b; Dutil et al., 2010) and also for quinolone resistant *E. coli* (Bortolia et al., 2010). Further insight into such dissemination might be used to prevent spread by minimizing occurrence in breeding stock coupled with infection control measures and relevant disinfection routines in hatcheries and in houses for rearing broilers.

TABLE H1 I. Number of samples with growth of *Escherichia coli* on McConkey-agar with 1 mg/L cefotaxime with transferable cefotaxime resistance.

Sampling period	Total number of positive isolates	Genotype of isolates	
		CTX-M-1	CMY-2
Spring (n=100)	32 (32%)	10 (10%)	22 (22%)
Autumn (n=100)	36 (36%)	2 (2%)	34 (34%)
Total	68 (34%)	12 (6%)	56 (28%)

TABLE H1 II. Resistance phenotypes and range of MICs of 68 *Escherichia coli* by genotype. Grey areas indicate MICs above EUCAST ECOFFs.

Genotype	n	Ctx	Am	Ci	Nal	Gm	Sm	Tc	Col	Ff	Cm	Km	Su	Tp
CMY-2	32	>2	128	0.03-0.06	≤1-4	0.5-1	4-8	≤1-2	<0.5-2	≤4-8	≤2-4	≤8	≤8-32	≤0.12-0.5
CMY-2	8	>2	64	0.12-0.5	32->128	0.5-4	8	≤1-2	<0.5-1	≤4-8	4	≤8	≤8-32	0.25-0.5
CMY-2	8	>2	64->128	0.03-0.06	2-4	0.5-1	4-16	≤1-2	<0.5-1	≤4-8	4-8	≤8	64->1024	0.25-1
CMY-2	3	>2	64	0.03	≤1-2	1-2	64	64->128	<0.5	≤4-8	4	>16	>1024	0.25
CMY-2	1	>2	128	0.12	64	1	8	128	<0.5	≤4	4	≤8	≤8	0.25
CMY-2	1	>2	128	0.25	128	0.5	8	<1	<0.5	≤4	≤2	≤8	>1024	≤0.12
CMY-2	1	>2	128	0.03	2	1	8	128	<0.5	≤4	4	≤8	≤8	0.25
CMY-2	1	>2	64	0.03	4	0.5	8	64	<0.5	16	4	≤8	64	0.5
CMY-2	1	>2	64	0.06	32	0.5	8	2	<0.5	≤4	4	≤8	16	0.25
CTX-M-1	9	>2	64->128	0.016-0.06	≤1-4	0.5-2	4-8	32-64	<0.5-1	≤4-8	≤2-8	≤8-16	>1024	0.25-0.5
CTX-M-1	3	>2	>128	0.03	2-4	0.5-1	32-128	32-128	<0.5-1	≤4	4	≤8	>1024	0.25-0.5



Indicator bacteria

RESISTANCE IN INDICATOR bacteria from broilers and from broiler meat was studied by culture of caecal content, collected at slaughter, and of retail broiler meat. Samples of caecal content were in addition screened for *Escherichia coli* resistant to third generation cephalosporins and for vancomycin resistant enterococci (VRE). Likewise samples of meat were screened for VRE. Also faeces from healthy horses were cultured for indicator bacteria and also screened for *E. coli* resistant to third generation cephalosporins. For details on methodology see Appendix 3.

Escherichia coli

Broilers

Escherichia coli were isolated from 181 (91%) of 200 samples cultured. The majority of the isolates (72%) were susceptible to all antimicrobials tested but 50 isolates (28%) were resistant to at least one substance (Table EC I). Resistance to quinolones (nalidixic acid and ciprofloxacin) was the most common trait (13%).

Fifteen isolates (8%) were resistant to two or more antimicrobials and 12 isolates (7%) were resistant to three or more antimicrobials. Phenotypes of multiresistant isolates are presented in Table EC II and associations between resistance traits, indicating linked resistance genes, in Table EC III.

Two isolates were resistant to cefotaxime and carried genes of CTX-M-1 type. In addition, on screening for resistance to third generation cephalosporins, 12 samples yielded *E. coli* with resistance of CTX-M-1 type and 56 samples isolates of CMY-2 type (for more details see Highlight *Escherichia coli* with ESBL- or transferable AmpC-type resistance in broilers).

Broiler meat

Escherichia coli were isolated from 77 (77%) of 100 samples cultured. The majority (65%) of isolates were susceptible to all antimicrobials tested but 27 isolates (35%) were resistant to at least one substance (Table EC I). Resistance to sulphamethoxazole was the most common trait (17%).

Six isolates (8%) were resistant to two or more antimicrobials and 2 isolates (3%) to three or more antimicrobials. The phenotypes of multiresistant isolates are presented in Table EC II.

Horses

Escherichia coli were isolated from 274 of 431 samples cultured. The majority (81%) of isolates were susceptible to all antimicrobials tested but 19 isolates (19%) were resistant to at least one substance (Table EC I). Resistance to trimethoprim was the most common trait (16%).

Forty-one isolates (15%) were resistant to two or more antimicrobials and 34 isolates (12%) were resistant to three or more antimicrobials. The phenotypes of multiresistant isolates are presented in Table EC II.

Comments

Broilers and broiler meat

Resistance in *E. coli* from broilers is low. This is in agreement with the rare use of antimicrobials effective against *E. coli* for treatment of broilers in Sweden where only small amounts of amoxicillin and minute amounts of enrofloxacin are used (see "Use of antimicrobials"). The low prevalence of resistance in the enteric flora of broilers is reflected by an equally low level in *E. coli* contaminating broiler meat. But notably, sulphonamide resistance in *E. coli* from broiler meat is more than twice as common as in *E. coli* from broilers. This could indicate other sources for contamination of broiler meat than enteric *E. coli* from slaughtered birds.

The prevalence of resistance to several antimicrobials is numerically higher in 2010 than in the most recent study in 2007 (Table EC I and Fig EC 1). However, the use of amoxicillin in broiler production years 2007-2009, due to outbreaks of botulism (see "Use of antimicrobials"), is not reflected by an increased frequency of ampicillin resistance. Most notably resistance to quinolones, i.e. nalidixic acid and ciprofloxacin, in *E. coli* from broilers has increased from 2% in 2002 to 13% 2010. Moreover, screening intestinal content by selective culture revealed *E. coli* with ESBL- or AmpC-type resistance in a large proportion of broilers. These findings are puzzling and cannot be explained by antimicrobial use in Swedish broiler production. More details are given in Highlight "*Escherichia coli* with ESBL- or transferable AmpC-type resistance in broilers".

Horses

Resistance to trimethoprim, sulphonamides or streptomycin in *E. coli* from horses are the dominating findings (Table EC I). Prevalence of resistance to these antimicrobials is higher than data from other animal species presented in previous SVARM reports. Resistance to trimethoprim and sulphonamides probably reflects the usage of these two substances in horses (see "Use of antimicrobials") while resistance to streptomycin is probably due to co-resistance with trimethoprim and sulphonamides. However, combination products with streptomycin could be used for instance uterine flushing solutions. The prevalence of resistance to several antimicrobials is shown in Table EC I. Eighty five percent of the isolates resistant to three or more antimicrobials were resistant to trimethoprim, sulphonamides and streptomycin.

Resistance to quinolones was rare and resistance to 3rd generation cephalosporins was not found in indicator *E. coli* from horses. However, when the fecal samples were cultured on selective media with cefotaxime ESBL producing *E. coli* was isolated. In total six faecal samples (1%) contained *E. coli* that produced SHV (see Highlight "*Enterobacteriaceae* producing extended spectrum beta-lactamases (ESBL) – isolates from diagnostic submissions").

TABLE EC I. Resistance and multiresistance (%) of *Escherichia coli* from broilers, broiler meat and horses, 2010. Data from previous SVARM-reports are included.

Antimicrobial	Cut-off value (mg/L)	Resistance (%) (95% confidence interval in brackets)							
		Broilers					Broiler meat	Horses	
		2000 n=274	2001 n=296	2002 n=306	2004 n=300	2007 n=296	2010 n=181	2010 n=77	2010-11 n=274
Ampicillin	>8	5 (2.6-8.0)	3 (1.2-5.3)	4 (2.3-7.2)	4 (2.1-6.9)	5 (2.6-7.8)	6 (3.1-10.6)	10 (4.6-19.4)	2 (0.4-3.7)
Cefotaxime	>0.25	-	-	-	-	1 (0.2-2.9)	1 (0.1-3.9)	0 (0.0-4.7)	0 (0.0-1.3)
Ceftiofur	>1	0 (0.0-1.3)	0 (0.0-1.2)	<1 (0.1-2.3)	0 (0.0-1.2)	1 (0.2-2.9)	-	-	-
Chloramphenicol	>16	<1 (0.1-2.6)	0 (0.0-1.2)	0 (0.0-1.2)	0 (0.0-1.2)	<1 (0.0-1.9)	0 (0.0-2.0)	1 (0.0-7.0)	<1 (0.0-2.0)
Ciprofloxacin	>0.06	4 ^b (2.3-7.5)	2 ^b (0.6-3.9)	4 ^b (2.3-7.2)	5 ^b (2.8-8.1)	7 (4.5-10.7)	13 (8.2-18.4)	6 (2.1-14.5)	<1 (0.0-2.0)
Colistin	>2	-	-	-	-	-	0 (0.0-2.0)	0 (0.0-4.7)	<1 (0.1-2.6)
Florfenicol	>16	0 (0.0-1.3)	0 (0.0-1.2)	0 (0.0-1.2)	0 (0.0-1.2)	0 (0.0-1.2)	0 (0.0-2.0)	0 (0.0-4.7)	0 (0.0-1.3)
Gentamicin	>2	<1 ^c (0.0-2.0)	<1 ^c (0.0-1.9)	<1 ^c (0.0-1.8)	<1 (0.1-2.4)	<1 (0.0-1.9)	0 (0.0-2.0)	0 (0.0-4.7)	<1 (0.1-2.6)
Kanamycin	>8	-	-	-	-	2 (0.6-3.9)	4 (1.9-8.5)	1 (0.0-7.0)	4 (2.3-7.5)
Nalidixic acid	>16	4 (2.3-7.5)	2 (0.6-3.9)	5 (2.5-7.6)	5 (2.8-8.1)	7 (4.2-10.3)	13 (8.2-18.4)	6 (2.1-14.5)	<1 (0.0-2.0)
Streptomycin	>16	5 (2.8-8.4)	6 (3.4-9.0)	5 (3.0-8.4)	6 (3.3-8.9)	4 (1.9-6.6)	7 (3.5-11.3)	4 (0.8-11.0)	13 (9.1-17.3)
Sulphonamide	>256	12 (8.1-16.0)	12 (8.4-16.1)	10 (6.7-13.7)	9 (6.0-12.8)	6 (3.7-9.5)	7 (3.5-11.3)	17 (9.3-27.1)	15 (10.9-19.7)
Tetracycline	>8	8 (4.8-11.5)	4 (2.4-7.4)	6 (3.3-8.8)	6 (3.6-9.3)	3 (1.6-6.1)	8 (4.3-12.6)	8 (2.9-16.2)	2 (0.6-4.2)
Trimethoprim	>2	<1 (0.1-2.6)	1 (0.4-3.4)	<1 (0.0-1.8)	<1 (0.1-2.4)	<1 (0.1-2.4)	3 (1.2-7.1)	1 (0.0-7.0)	16 (11.9-20.9)
Multiresistance^a									
Susceptible to all		77	78	77	84	85	72	65	81
Resistant to 1		15	17	16	9	10	19	27	4
Resistant to 2		5	3	4	2	1	2	5	3
Resistant to 3		2	1	1	2	1	3	1	9
Resistant to >3		1	2	1	3	3	3	1	3

^a Enrofloxacin/ciprofloxacin/nalidixic acid as well as cefotaxime/ceftiofur considered as one substance; ^b Enrofloxacin tested, cut-off value >0.12mg/L;

^c Cut-off value >4 mg/L.

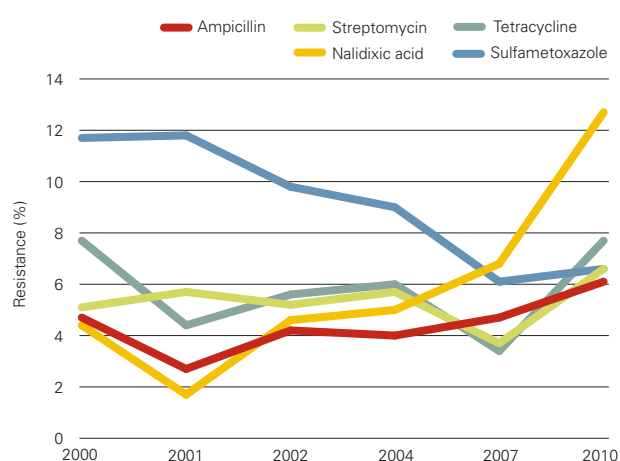
FIGURE EC I. Resistance in *Escherichia coli* from broilers 2000-2010.

TABLE EC II. Phenotypes of multiresistant *Escherichia coli* from broilers, broiler meat and horses, 2010. "R" in shaded fields indicates resistance. Data from previous SVARM-reports are included.

Broilers 2000-07 n=1472	Broilers 2010 n=181	Broiler meat 2010 n=77	Horses 2010-11 n=274	Resistance phenotype										
				Su	Tc	Sm	Nal	Ci/Ef	Am	Tm	Cm	Gm	Km	Ctx/Ce
2				R	R	R	R	R	S	R	S	S	S	S
1				R	R	R	R	R	S	S	R	S	S	S
16				R	R	R	R	R	S	S	S	S	S	S
1				R	R	R	R	S	R	S	S	S	S	S
2				R	R	R	R	S	S	S	S	S	S	S
1				R	R	R	S	S	R	R	S	S	S	S
2	1			R	R	R	S	S	R	S	S	S	S	S
	1			R	R	R	S	S	R	S	S	S		R
1			1	R	R	R	S	S	R	R	S	R	R	S
	1		3	R	R	R	S	S	S	R	S	S	S	S
5				R	R	R	S	S	S	S	S	S	S	S
2				R	R	S	R	R	S	S	S	S	S	S
1				R	R	S	R	S	S	S	S	S	S	S
	1			R	R	S	S	S	R	S	S	S		R
1				R	R	S	S	S	S	S	S	R		S
	1			R	S	R	R	R	R	S	S	S	S	S
1	1			R	S	R	R	R	S	S	S	S	S	S
1				R	S	R	R	R	S	S	S	R		S
1		1		R	S	R	S	S	R	R	S	S	S	S
1				R	S	R	S	S	R	S	R	S		S
2				R	S	R	S	S	S	S	S	R		S
1				R	S	S	S	S	R	S	S	R		S
			3	R	S	S	S	S	S	R	S	S	R	S
1				S	R	R	R	R	S	S	S	S	S	S
1	4			S	R	R	S	S	R	S	S	S	S	S
1				S	R	R	S	S	S	S	S	R		S
1				S	R	S	R	R	R	S	S	S	S	S
1	2			S	R	S	S	R	R	S	S	S	S	S
1				S	R	S	S	S	R	S	S	S		R
1		1		S	S	S	R	R	R	S	R	S	S	S
1				S	S	S	R	R	S	R	S	R		S
			23	R	S	R	S	S	S	R	S	S	S	S
			1	R	S	R	S	S	S	R			R	S
			1	R	S	S	S	R	R	R	S	S	S	S
			1	R	S	R	S	S	S	R	S	R		S
				R	S	R	S	S	R	R	S	R		S
			1	R	S	R	S	S	R	R	R	R	R	S
50 (3.4%)	12 (6.6%)	2 (3.0%)	34 (12%)	Number of isolates (percent of all isolates)										

TABLE EC III. Association between resistance traits in *Escherichia coli* from broilers 2000-2010. For each antimicrobial the first row gives prevalence of resistance to other antimicrobials in susceptible isolates (S) and the second row prevalence in resistant isolates (R). All antimicrobials were not tested each year and all combinations of resistance traits can therefore not be calculated.

Single substance susceptibility		n	Resistance (%)								
			Am	Cm	Ff	Gm	Nal	Sm	Su	Tc	Tm
Ampicillin	S	1582	0.0	0.1	0.0	1.8	5.4	4.5	8.7	4.6	0.9
	R	71	100	2.8	0.0	1.4	5.6	22.5	23.9	25.4	2.8
Apramycin	S	876	3.9	0.2	0.0	3.1	3.5	5.4	11.1	5.8	0.8
	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cefotaxime	S	472	4.2	0.2	0.0	0.2	9.1	4.7	5.9	4.0	1.7
	R	5	100	0.0	0.0	0.0	0.0	20.0	40.0	40.0	0.0
Ceftiofur	S	1467	3.7	0.2	0.0	2.0	4.5	5.1	9.7	5.3	0.7
	R	5	100	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0
Chloramph	S	1650	4.2	0.0	0.0	1.8	5.3	5.2	9.2	5.4	1.0
	R	3	66.7	100	0.0	0.0	66.7	66.7	66.7	33.3	0.0
Ciprofloxacin	S	433	5.1	0.0	0.0	0.0	0.0	3.2	4.8	3.2	1.8
	R	44	6.8	2.3	0.0	2.3	97.7	20.5	20.5	15.9	0.0
Colistin	S	181	6.1	0.0	0.0	0.0	12.7	6.6	6.6	6.1	3.3
	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Enrofloxacin	S	1131	4.0	0.1	0.0	2.4	0.4	4.0	9.5	4.3	0.5
	R	45	2.2	2.2	0.0	4.4	93.3	42.2	35.6	44.4	6.7
Florfenicol	S	1653	4.3	0.2	0.0	1.8	5.4	5.3	9.3	5.4	1.0
	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	S	1623	4.3	0.2	0.0	0.0	5.3	5.1	9.1	5.4	0.9
	R	30	3.3	0.0	0.0	100	10.0	16.7	23.3	10.0	6.7
Kanamycin	S	464	5.2	0.2	0.0	0.2	7.5	3.2	4.3	3.2	1.7
	R	13	7.7	0.0	0.0	0.0	61.5	61.5	76.9	46.2	0.0
Nalidixic acid	S	1564	4.3	0.1	0.0	1.7	0.0	3.6	8.0	3.8	0.9
	R	89	4.5	2.2	0.0	3.4	100	34.8	32.6	33.7	3.4
Neomycin	S	1157	3.8	0.2	0.0	2.5	2.6	4.0	9.4	4.7	0.7
	R	19	10.5	0.0	0.0	0.0	84.2	94.7	78.9	78.9	5.3
Streptomycin	S	1566	3.5	0.1	0.0	1.6	3.7	0.0	6.5	3.1	0.7
	R	87	18.4	2.3	0.0	5.7	35.6	100	59.8	48.3	6.9
Sulphamethox.	S	1499	3.6	0.1	0.0	1.5	4.0	2.3	0.0	2.5	0.6
	R	154	11.0	1.3	0.0	4.5	18.8	33.8	100	33.8	5.2
Tetracycline	S	1563	3.4	0.1	0.0	1.7	3.8	2.9	6.5	0.0	0.8
	R	90	20.0	1.1	0.0	3.3	33.3	46.7	57.8	100	5.6
Trimethoprim	S	1636	4.2	0.2	0.0	1.7	5.3	5.0	8.9	5.2	0.0
	R	17	11.8	0.0	0.0	11.8	17.6	35.3	47.1	29.4	100

Enterococcus

Broilers

From 200 samples of caecal content of broilers, 35 isolates of *Enterococcus faecalis* and 136 isolates of *E. faecium* were obtained. In both species resistance to narasin, erythromycin, tetracycline and bacitracin were the most common traits (Table ENT I & II). Multiresistance was rare in both species but commonly involved these antimicrobials which also often are associated in the same isolate (Table ENT I, II, III & IV).

Vancomycin resistant enterococci (VRE) were isolated from 46 (23%) of 200 samples screened by culture on vancomycin supplemented media (16 mg/L) (Fig ENT I). All 46 isolates were *E. faecium* with MIC for vancomycin >128 mg/L and 16 isolates examined by molecular methods all carried the *vanA*-gene.

Broiler meat

From 100 samples of retail broiler meat 81 isolates of *E. faecalis* and 17 of *E. faecium* were obtained. Resistance to narasin, erythromycin, tetracycline and bacitracin were the most common traits in *E. faecalis* (Table ENT I) whereas narasin resistance was predominant in *E. faecium* (Table ENT II). No isolate from meat was resistant to more than two antimicrobials. Vancomycin resistant enterococci (VRE) were isolated from 2 (2%) of 100 samples screened by culture on vancomycin supplemented media (16 mg/L) (Fig ENT I). Both isolates were *E. faecium* with MIC for vancomycin >128 mg/L.

Horses

From faecal samples of 431 horses, 34 isolates of *E. faecalis* and 27 isolates of *E. faecium* were obtained. In *E. faecalis*, resistance to tetracycline was the most common trait (44%). Multiresistance occurred in six isolates of *E. faecalis* (18%) and these isolates were resistant to erythromycin, gentamicin, kanamycin, tetracycline and chloramphenicol. Two of these isolates were also resistant to streptomycin.

Resistance was rare in *E. faecium*. Only one isolate was resistant to two antimicrobials; ampicillin and streptomycin.

Comments

Broilers and broiler meat

There are no obvious or untoward trends in resistance in enterococci from broilers. The prevalence of resistance in both *E. faecalis* and *E. faecium* has been stable or declining since first studied in 2000 (Table I & II). For example, tetracycline resistance in *E. faecium* has gradually declined from 39% in 2000 to 12% in 2010. Notably the use of amoxicillin in broiler produc-

tion in the years 2007-2009 due to outbreaks of botulism is not reflected in an increased occurrence of resistance in enterococci (see "Use of antimicrobials").

Resistance in enterococci from broilers is roughly reflected by resistance in enterococci from broiler meat. The same resistance traits are found but for example resistance to narasin or erythromycin in *E. faecalis* is less common in isolates from meat than in isolates from broilers (Table ENT I). Also the proportion of *E. faecalis* to *E. faecium* is different in broiler meat compared to in broilers (Table ENT I & II). In broiler meat, *E. faecalis* is more common than *E. faecium* whereas the opposite applies for broilers. This indicates that enterococci on broiler meat might partly emanate from other sources than the intestinal flora of broilers.

The prevalence of VRE among broilers, screened by culture on vancomycin supplemented media, gradually increased from less than one percent in 2000 to a peak of 41% in 99 samples cultured in 2005 (Figure ENT I). The increase was caused by spread of a single clone of *E. faecium* carrying the *vanA* gene (Nilsson et al., 2009). The increase has abated but VRE still prevail at a stable level of around 25 % of samples cultured (Figure ENT I). Notably VRE of the *vanA* genotype were isolated from two samples of broiler meat.

Horses

The most notable finding in *E. faecalis* from horses was a quadruple resistance; macrolides, aminoglycosides (gentamicin and kanamycin), tetracyclines and chloramphenicol found in six isolates (18%). Clonal relationships between these enterococci are to be further investigated.

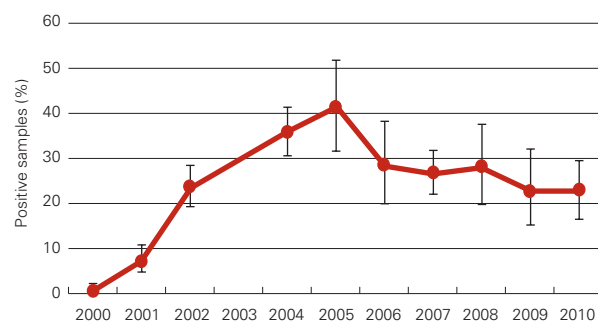


FIGURE ENT I. Proportion (%) samples of intestinal content from healthy broilers positive for VRE when cultured on vancomycin supplemented media (16 mg/L), 95% confidence intervals indicated. Number of samples cultured each year was between 99 and 351.

TABLE ENT I. Percent resistance and multiresistance of *Enterococcus faecalis* from broiler chickens, broiler meat and horses, 2010. Data from previous SVARM-reports are included.

Antimicrobial	Cut-off value (mg/L)	Resistance (%) (95% confidence interval in brackets)							
		Broilers					Broiler meat	Horses	
		2000 n=47	2001 n=49	2002 n=57	2004 n=48	2007 n=28	2010 n=35	2010 n=81	2010-11 n=34
Ampicillin	>4	0 (0.0-7.5)	0 (0.0-7.3)	0 (0.0-6.3)	0 (0.0-7.4)	0 (0.0-12.3)	0 (0.0-10.0)	0 (0.0-4.5)	0 (0.0-10.3)
Bacitracin	>32	23 (12.3-38.0)	31 (18.3-45.4)	35 (22.9-48.9)	29 (17.0-44.1)	11 (2.3-28.2)	14 (4.8-30.3)	15 (7.9-24.4)	0 (0.0-10.3)
Chloramph.	>32	-	-	-	0 (0.0-7.4)	0 (0.0-12.3)	0 (0.0-10.0)	0 (0.0-4.5)	18 (6.8-34.5)
Erythromycin	>4	30 (17.3-44.9)	41 (27.0-55.8)	26 (15.5-39.7)	25 (13.6-39.6)	29 (13.2-48.7)	31 (16.9-49.3)	23 (14.8-34.2)	21 (8.7-37.9)
Gentamicin	>32	0 (0.0-7.5)	0 (0.0-7.3)	2 (0.0-9.4)	0 (0.0-7.4)	0 (0.0-12.3)	0 (0.0-10.0)	0 (0.0-4.5)	21 (8.7-37.9)
Kanamycin	>1024	-	-	-	-	4 (0.1-18.3)	3 (0.1-14.9)	0 (0.0-4.5)	21 (8.7-37.9)
Linezolid	>4	-	-	-	-	0 (0.0-12.3)	0 (0.0-10.0)	0 (0.0-4.5)	0 (0.0-10.3)
Narasin	>2	43 (28.3-57.8)	45 (30.7-59.8)	39 (26.0-52.4)	35 (22.2-50.5)	36 (18.6-55.9)	37 (21.5-55.1)	19 (10.8-28.7)	0 (0.0-10.3)
Streptomycin	>512	9 (2.4-20.4)	6 (1.3-16.9)	11 (4.0-21.5)	4 (0.5-14.3)	0 (0.0-12.3)	0 (0.0-10.0)	4 (0.8-10.4)	9 (1.9-23.7)
Tetracycline	>4	60 (44.3-73.6)	67 (52.5-80.1)	61 (47.2-74.0)	48 (31.4-60.8)	57 (37.2-75.5)	31 (16.9-49.3)	37 (26.6-48.5)	44 (27.2-62.1)
Vancomycin	>4	0 (0.0-7.5)	0 (0.0-7.3)	0 (0.0-6.3)	0 (0.0-7.4)	0 (0.0-12.3)	0 (0.0-10.0)	0 (0.0-4.5)	0 (0.0-10.3)
Virginiamycin	>32	0 (0.0-7.5)	0 (0.0-7.3)	0 (0.0-6.3)	0 (0.0-7.4)	0 (0.0-12.3)	0 (0.0-10.0)	0 (0.0-4.5)	0 (0.0-10.3)
Multiresistance (%)									
Susceptible to all above		15	10	18	23	25	31	30	56
Resistant to 1		47	33	28	29	36	34	43	24
Resistant to 2		13	29	35	35	21	23	27	0
Resistant to 3		15	14	7	8	14	9		0
Resistant to >3		11	14	12	4	4	3		21

TABLE ENT II. Percent resistance and multiresistance of *Enterococcus faecium* from broiler chickens, broiler meat and horses, 2010. Previous data from SVARM included.

Antimicrobial	Cut-off value (mg/L)	Resistance (%) (95% confidence interval in brackets)							
		Broilers					Broiler meat	Horses	
		2000 n=151	2001 n=204	2002 n=189	2004 n=163	2007 n=197	2010 n=136	2010 n=17	2010-11 n=27
Ampicillin	>4	8 (4.2-13.5)	5 (2.4-8.8)	4 (1.8-8.2)	2 (0.7-6.2)	1 (0.1-3.6)	2 (0.5-6.3)	0 (0.0-19.5)	14 (4.2-33.7)
Bacitracin	>32	20 (13.8-27.1)	15 (10.1-20.3)	24 (18.4-31.1)	32 (24.8-39.6)	23 (17.2-29.3)	15 (9.8-22.6)	18 (3.8-43.4)	0 (0-12.8)
Chloramph.	>32	-	-	-	0 (0.0-2.2)	0 (0.0-1.9)	0 (0.0-2.7)	0 (0.0-19.5)	0 (0-12.8)
Erythromycin	>4	12 (7.2-18.2)	15 (10.1-20.3)	11 (7.0-16.5)	10 (5.7-15.5)	11 (7.1-16.4)	13 (8.0-20.1)	6 (0.1-28.7)	0 (0-12.8)
Gentamicin	>32	0 (0.0-2.4)	0 (0.0-1.8)	0 (0.0-1.9)	0 (0.0-2.2)	0 (0.0-1.9)	0 (0.0-2.7)	0 (0.0-19.5)	0 (0-12.8)
Kanamycin	>1024	-	-	-	-	0 (0.0-1.9)	0 (0.0-2.7)	0 (0.0-19.5)	0 (0-12.8)
Linezolid	>4	-	-	-	-	0 (0.0-1.9)	0 (0.0-2.7)	0 (0.0-19.5)	0 (0-12.8)
Narasin	>2	80 (72.1-85.6)	80 (73.7-85.2)	78 (71.2-83.5)	93 (88.2-96.6)	89 (83.6-92.9)	91 (85.1-95.4)	94 (71.3-99.9)	0 (0-12.8)
Streptomycin	>512	<1 (0.0-3.6)	0 (0.0-1.8)	0 (0.0-1.9)	<1 (0.0-3.4)	<1 (0.1-2.8)	0 (0.0-2.7)	0 (0.0-19.5)	4 (0.1-19.0)
Tetracycline	>4	39 (32.2-47.3)	28 (22.4-35.2)	25 (19.4-32.2)	18 (12.8-25.2)	14 (9.7-19.9)	12 (7.5-19.3)	0 (0.0-19.5)	4 (0.1-19.0)
Vancomycin	>4	1 ^a (0.2-4.7)	0 (0.0-1.8)	1 (0.1-3.8)	2 (0.4-5.3)	0 (0.0-1.9)	0 (0.0-2.7)	0 (0.0-19.5)	0 (0-12.8)
Virginiamycin	>4	24 (17.9-32.2)	22 (16.6-28.4)	24 (18.4-31.1)	9 (5.2-14.7)	4 (1.8-7.8)	5 (2.1-10.3)	6 (0.1-28.7)	4 (0.1-19.0)
Multiresistance (%)									
Susceptible to all above		11	10	13	3	9	4	0	78
Resistant to 1		28	35	30	41	48	60	77	18
Resistant to 2		36	38	38	44	37	27	23	4
Resistant to 3		18	15	17	10	7	7		0
Resistant to >3		7	2	3	2	1	1		0

^a Isolates with MIC 8 mg/L.

TABLE ENT III. Phenotypes of *Enterococcus faecalis* resistant to three or more antimicrobials and of *Enterococcus faecium* resistant to four or more antimicrobials. Broilers, 2010. "R" in shaded fields indicates resistance. Previous data from SVARM included.

<i>E. faecalis</i>								<i>E. faecium</i>									
Year		Resistance phenotype						Year		Resistance phenotype							
2000-07 n=201	2010 n=35	Em	Na	Ba	Tc	Sm	Km	2000-07 n=904	2010 n=136	Na	Tc	Ba	Vi	Em	Am	Sm	Va
5		R	R	R	R	R		1		R	R	R	R			R	
14	1	R	R	R	R			2		R	R	R	R	R			
1		R	R	R			R	1		R	R	R	R				R
3		R	R	R				6		R	R	R	R				
1		R	R				R	2		R	R	R			R		
10	2	R	R		R			2		R	R	R		R			
1		R	R		R	R		2		R	R	R				R	
7		R		R	R			1		R	R		R	R	R		
1	1	R			R		R	2		R	R		R		R		
1		R			R	R		5	1	R	R		R	R			
3			R	R	R			2		R	R			R	R		
1			R	R		R		1		R		R	R				R
								1		R			R	R	R		
48 (21%)	4 (11%)	Number of isolates (percent of all isolates)						28 (3%)	1 (<1%)	Number of isolates (percent of all isolates)							

TABLE ENT IV. Association between resistance traits in *Enterococcus faecalis* and in *Enterococcus faecium* from broilers 2000-10. For each antimicrobial the first row gives prevalence of resistance to other antimicrobials in susceptible isolates (S) and the second row prevalence in resistant isolates (R). All antimicrobials were not tested each year and all combinations of resistance traits can therefore not be calculated.

Single substance susceptibility	<i>E. faecalis</i>											<i>E. faecium</i>										
	n	Cross resistance (%)										n	Cross resistance (%)									
		Am	Ba	Em	Gm	Na	Sm	Tc	Va	Vi	Am		Ba	Em	Gm	Na	Sm	Tc	Va	Vi		
Ampicillin	S	264	0.0	25.8	30.3	0.0	39.4	5.7	55.3	0.0	0.0	S	1001	0.0	21.2	12.0	0.0	85.2	0.3	22.5	0.7	15.2
	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	R	39	100	30.8	12.8	0.0	71.8	0.0	38.5	0.0	15.4
Bacitracin	S	196	0.0	0.0	25.0	0.0	38.3	4.1	45.4	0.0	0.0	S	816	3.3	0.0	13.5	0.0	82.7	0.2	24.6	0.6	16.7
	R	68	0.0	100	45.6	0.0	42.6	10.3	83.8	0.0	0.0	R	224	5.4	100	6.7	0.0	92.0	0.4	17.4	0.9	9.8
Chloramph.	S	111	0.0	19.8	27.9	0.0	36.0	1.8	45.0	0.0	0.0	S	496	1.8	23.8	11.3	0.0	90.9	0.4	15.1	0.6	6.0
	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erythromycin	S	184	0.0	20.1	0.0	0.0	28.3	3.3	51.6	0.0	0.0	S	915	3.7	22.8	0.0	0.0	84.3	0.2	22.0	0.3	15.6
	R	80	0.0	38.8	100	0.0	65.0	11.3	63.8	0.0	0.0	R	125	4.0	12.0	100	0.0	88.0	0.8	31.2	3.2	12.0
Gentamicin	S	264	0.0	25.8	30.3	0.0	39.4	5.7	55.3	0.0	0.0	S	1040	3.8	21.5	12.0	0.0	84.7	0.3	23.1	0.7	15.2
	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Kanamycin	S	61	0.0	13.1	27.9	0.0	37.7	0.0	41.0	0.0	0.0	S	333	1.5	19.8	12.0	0.0	89.8	0.3	13.5	0.0	4.5
	R	2	0.0	0.0	100	0.0	0.0	0.0	100	0.0	0.0	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Linezolid	S	63	0.0	12.7	30.2	0.0	36.5	0.0	42.9	0.0	0.0	S	333	1.5	19.8	12.0	0.0	89.8	0.3	13.5	0.0	4.5
	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Narasin	S	160	0.0	24.4	17.5	0.0	0.0	3.8	56.9	0.0	0.0	S	159	6.9	11.3	9.4	0.0	0.0	1.3	22.0	0.0	8.2
	R	104	0.0	27.9	50.0	0.0	100	8.7	52.9	0.0	0.0	R	881	3.2	23.4	12.5	0.0	100	0.1	23.3	0.8	16.5
Streptomycin	S	249	0.0	24.5	28.5	0.0	38.2	0.0	55.0	0.0	0.0	S	1037	3.8	21.5	12.0	0.0	84.9	0.0	23.0	0.7	15.1
	R	15	0.0	46.7	60.0	0.0	60.0	100	60.0	0.0	0.0	R	3	0.0	33.3	33.3	0.0	33.3	100	33.3	0.0	33.3
Tetracycline	S	118	0.0	9.3	24.6	0.0	41.5	5.1	0.0	0.0	0.0	S	800	3.0	23.1	10.8	0.0	84.5	0.3	0.0	0.6	11.4
	R	146	0.0	39.0	34.9	0.0	37.7	6.2	100	0.0	0.0	R	240	6.3	16.3	16.3	0.0	85.4	0.4	100	0.8	27.9
Vancomycin	S	264	0.0	25.8	30.3	0.0	39.4	5.7	55.3	0.0	0.0	S	1033	3.8	21.5	11.7	0.0	84.6	0.3	23.0	0.0	15.1
	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	R	7	0.0	28.6	57.1	0.0	100	0.0	28.6	100	28.6
Virginiamycin	S	264	0.0	25.8	30.3	0.0	39.4	5.7	55.3	0.0	0.0	S	882	3.7	22.9	12.5	0.0	83.4	0.2	19.6	0.6	0.0
	R	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	R	158	3.8	13.9	9.5	0.0	91.8	0.6	42.4	1.3	100

TABLE EC IV. Percent resistance (R) and distribution of MICs for *Escherichia coli* from horses (n=272), broilers (n=181) and broiler meat (n=77), 2010. Previous data from broiler, SVARM 2000-07 included (n=1472).

Antimicrobial	Year/ source	R (%)	Distribution (%) of MICs (mg/L)																	
			≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Ampicillin	2010 Horses	2								4.7	46.7	45.6	1.1	0.4						1.5
	2010 Broilers	6								9.9	70.2	12.7	1.1						6.1	
	2010 Meat	10								2.6	70.1	16.9					2.6		7.8	
	2000-07 Broilers	4							0.4	7.4	50.1	36.8	1.2	0.1	0.1	3.9				
Cefotaxime	2010 Horses	0		0.4	1.8	62.8	33.9	1.1												
	2010 Broilers	1			3.3	60.8	32.6	2.2			1.1									
	2010 Meat	0		1.3	1.3	67.5	29.9													
	2000-07 Broilers	1 ^a				52.0	37.5	9.5			1.0									
Chloramph.	2010 Horses	<1								6.2	0.4	69.3	23.7					0.4		
	2010 Broilers	0								9.4	60.2	30.4								
	2010 Meat	1								7.8	51.9	39.0							1.3	
	2000-07 Broilers	<1								3.8	62.4	33.6	0.1	0.2						
Ciprofloxacin	2010 Horses	<1		5.5	84.3	9.9			0.4											
	2010 Broilers	13		5.5	61.3	20.4	1.7	7.7	3.3											
	2010 Meat	7	1.3	1.3	83.1	7.8	1.3	5.2												
	2000-07 Broilers	7 ^a		2.4	38.2	52.4	1.0	3.4	2.7											
Colistin	2010 Horses	0							53.3	44.2	1.8	0.7								
	2010 Broilers	0							57.5	37.0	5.5									
	2010 Meat	0							53.2	46.8										
	2000-07 Broilers	-																		
Florfenicol	2010 Horses	0									35.8	62.4	2.6							
	2010 Broilers	0									44.2	54.7	1.1							
	2010 Meat	0									32.5	63.6	3.9							
	2000-07 Broilers	0									43.1	55.6	1.4							
Gentamicin	2010 Horses	<1					2.6	56.6	37.2	2.4			0.4	0.4						
	2010 Broilers	0					1.7	40.9	49.2	8.3										
	2010 Meat	0					2.6	79.2	16.9	1.3										
	2000-07 Broilers	2						6.4	40.6	36.7	14.4	1.7	0.1	0.1						
Kanamycin	2010 Horses	4										95.6	3.6	0.7						
	2010 Broilers	4										95.6	2.2	2.2						
	2010 Meat	1										98.7	1.3							
	2000-07 Broilers	2 ^a								12.5	72.3	13.5		1.7						
Nalidixic acid	2010 Horses	<1							2.9	36.1	58.0	2.2	0.4						0.4	
	2010 Broilers	13							3.3	20.4	62.4	1.1		1.1	2.2	6.6	2.8			
	2010 Meat	7							2.6	23.4	64.9	2.6			1.3	1.3	3.9			
	2000-07 Broilers	5							0.6	19.0	53.0	21.6	1.3	0.1	1.2	1.4	1.9			
Streptomycin	2010 Horses	14								3.6	30.7	46.7	5.1	2.6	1.1	3.3	5.8	1.1		
	2010 Broilers	7								0.6	26.5	61.3	5.0	0.6	3.3	2.2	0.6			
	2010 Meat	4								6.5	51.9	36.4	1.3		2.6		1.3			
	2000-07 Broilers	5								0.1	11.5	57.5	25.7	1.4	0.8	1.2	1.1	0.6		
Sulphonamide	2010 Horses	15										12.0	33.2	34.3	5.5					15.0
	2010 Broilers	7										18.8	47.0	23.8	3.9					6.6
	2010 Meat	17										6.5	19.5	50.6	6.5					16.9
	2000-07 Broilers	10													69.2	20.7	0.5		9.6	
Tetracycline	2010 Horses	2							60.6	36.9	0.7				0.7	1.1				
	2010 Broilers	8							63.5	28.7				0.6	2.2	3.3	1.7			
	2010 Meat	8							54.5	37.7					3.9	2.6		1.3		
	2000-07 Broilers	5						0.7	31.9	51.8	9.7	0.4	0.1	0.1	0.3	5.0				
Trimethoprim	2010 Horses	16				5.1	36.5	36.9	5.5		0.4				15.7					
	2010 Broilers	3				5.0	30.9	42.0	18.2	0.6				0.6	2.8					
	2010 Meat	1				2.6	29.9	57.1	9.1						1.3					
	2000-07 Broilers	<1					21.9	53.9	21.5	2.0	0.1				0.6					

^a Data from 2007, n=296.

TABLE ENT V. Percent resistance (R) and distribution of MICs for *Enterococcus faecalis* from horses (n=34), broilers (n=35) and broiler meat (n=81), 2010. Previous data from SVARM 2000-07 included (n=229).

Antimicrobial	Year/ source	R (%)	Distribution (%) of MICs (mg/L)															
			≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Ampicillin	2010 Horses	0			5.9	82.4	11.8											
	2010 Broilers	0			5.7	91.4	2.9											
	2010 Meat	0			2.5	93.8	3.7											
	2000-07 Broilers	0		1.7	11.4	79.0	7.4	0.4										
Bacitracina	2010 Horses	0						2.9	64.7	29.4	2.9							
	2010 Broilers	14				2.9	2.9	17.1	40.0	22.9		5.7	8.6					
	2010 Meat	15					1.2	8.6	54.3	21.0		6.2	8.6					
	2000-07 Broilers	28				3.1	0.9	7.4	27.1	28.4	5.7	27.5						
Chloramphenicol	2010 Horses	21						20.6	58.8			17.6	2.9					
	2010 Broilers	0					2.9	22.9	74.3									
	2010 Meat	0						28.4	69.1	2.5								
	2000-07 Broilers	0 ^b						35.5	61.8	2.6								
Erythromycin	2010 Horses	21			5.9	29.4	26.5	17.6	20.6									
	2010 Broilers	31			22.9	17.1	28.6		14.3	5.7			11.4					
	2010 Meat	23			40.7	19.8	14.8	1.2	11.1	3.7			8.6					
	2000-07 Broilers	30			26.2	16.6	22.7	4.4	7.0	6.1	1.7	15.3						
Gentamicin	2010 Horses	21						2.9	14.7	55.9	5.9				20.6			
	2010 Broilers	0						2.9	54.3	42.9								
	2010 Meat	0							56.8	40.7	2.5							
	2000-07 Broilers	<1						2.6	6.1	42.4	41.0	7.4	0.4					
Kanamycin	2010 Horses	21										64.7	14.7					20.6
	2010 Broilers	3									14.3	80.0		2.9				2.9
	2010 Meat	0									13.6	81.5	4.9					
	2000-07 Broilers	4 ^c									32.1	60.7	3.6					3.6
Linezolid	2010 Horses	0					94.1	5.9										
	2010 Broilers	0			2.9	34.3	62.9											
	2010 Meat	0				12.3	86.4	1.2										
	2000-07 Broilers	0 ^c			3.6	3.6	85.7	7.1										
Narasin	2010 Horses	0	2.9	64.7	23.5	8.8												
	2010 Broilers	37	5.7	28.6	11.4	8.6	8.6	25.7	11.4									
	2010 Meat	19		39.5	23.5	2.5	16.0	9.9	8.6									
	2000-07 Broilers	40	4.4	24.0	22.7	2.2	7.0	23.1	14.0	2.2	0.4							
Streptomycin	2010 Horses	9									2.9	20.6	67.6		8.8			
	2010 Broilers	0										51.4	45.7	2.9				
	2010 Meat	4									1.2	48.1	44.4	2.5	2.5	1.2		
	2000-07 Broilers	7									11.8	52.8	28.8		1.3	5.2		
Tetracycline	2010 Horses	44			20.6	32.4	2.9				14.7	26.5	2.9					
	2010 Broilers	31			31.4	37.1					14.3	17.1						
	2010 Meat	37			13.6	44.4	1.2	3.7			7.4	24.7	4.9					
	2000-07 Broilers	59			9.6	24.5	6.1	0.9	1.3	15.3	19.2	23.1						
Vancomycin	2010 Horses	0				2.9	82.4	14.7										
	2010 Broilers	0				5.7	54.3	40.0										
	2010 Meat	0				1.2	53.1	45.7										
	2000-07 Broilers	0				13.5	71.6	14.8										
Virginiamycin	2010 Horses	0			2.9	5.9	2.9	2.9		47.1	38.2							
	2010 Broilers	0					2.9	2.9	11.4	77.1	5.7							
	2010 Meat	0			1.2	1.2		1.2	2.5	38.3	55.6							
	2000-07 Broilers	0			1.7	1.3	4.4	9.6	25.3	51.5	6.1							

^a MIC in U/mL, see Appendix 3 for details; ^b Data from 2004 and 2007, n=76; ^c Data from 2007, n=28.

TABLE ENT VI. Percent resistance (R) and distribution of MICs for *Enterococcus faecium* from horses (n=27), broilers (n=136) and broiler meat (n=17), 2010. Previous data from SVARM 2000-07 included (n=904).

Antimicrobial	Year/ source	R (%)	Distribution (%) of MICs (mg/L)															
			≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Ampicillin	2010 Horses	15		3.7	3.7	37.0	33.3	7.4	14.8									
	2010 Broilers	2		14.0	32.4	16.2	21.3	14.0	2.2									
	2010 Meat	0		11.8	35.3	29.4	17.6	5.9										
	2000-07 Broilers	4		10.5	18.9	24.8	28.2	13.6	3.9	0.1								
Bacitracin ^a	2010 Horses	0				7.4		3.7	22.2	40.7	25.9							
	2010 Broilers	15				34.6	10.3	8.8	19.1	8.1	3.7	8.8	2.2	4.4				
	2010 Meat	18				29.4	23.5	5.9	5.9	17.6		5.9	11.8					
	2000-07 Broilers	22				21.9	4.3	4.1	16.2	20.8	10.3	22.5						
Chloramphenicol	2010 Horses	0						29.6	70.4									
	2010 Broilers	0			0.7	3.7	64.0	30.1	0.7	0.7								
	2010 Meat	0				5.9	82.4	11.8										
	2000-07 Broilers	0 ^b				1.9	65.3	31.1	1.4	0.3								
Erythromycin	2010 Horses	0		29.6	29.6	29.6	11.1											
	2010 Broilers	13		33.8	41.9	8.8	2.2	4.4	2.2	0.7		5.9						
	2010 Meat	6		58.8	35.3			5.9										
	2000-07 Broilers	12		26.8	32.0	22.5	7.0	2.4	1.9	0.6	7.0							
Gentamicin	2010 Horses	0					11.1	85.2	3.7									
	2010 Broilers	0				1.5	14.0	69.1	14.7	0.7								
	2010 Meat	0					29.4	58.8	11.8									
	2000-07 Broilers	0				4.5	28.3	51.5	14.5	1.1								
Kanamycin	2010 Horses	0								25.9	3.7	63.0	7.4					
	2010 Broilers	0								2.2	15.4	47.8	25.7	6.6	2.2			
	2010 Meat	0								5.9	11.8	41.2	41.2					
	2000-07 Broilers	0 ^c								1.0	28.9	49.2	17.3	3.0	0.5			
Linezolid	2010 Horses	0				48.1	51.9											
	2010 Broilers	0		1.5	3.7	80.9	14.0											
	2010 Meat	0			5.9	94.1												
	2000-07 Broilers	0 ^c			5.1	80.7	14.2											
Narasin	2010 Horses	0		81.5	18.5													
	2010 Broilers	91		2.9	1.5	4.4	42.6	48.5										
	2010 Meat	94		5.9			52.9	41.2										
	2000-07 Broilers	84	0.2	0.8	5.0	4.4	5.9	35.4	45.8	2.5								
Streptomycin	2010 Horses	4									85.2	7.4	3.7				3.7	
	2010 Broilers	0								22.1	72.8	5.1						
	2010 Meat	0								35.3	64.7							
	2000-07 Broilers	<0								57.2	40.5	2.0			0.1	0.2		
Tetracycline	2010 Horses	0		29.6	63.0	3.7	3.7											
	2010 Broilers	12		76.5	9.6	0.7	0.7	2.9		2.9	5.9	0.7						
	2010 Meat	0		64.7	35.3													
	2000-07 Broilers	25		35.3	34.3	4.6	1.1	1.4	4.1	6.9	12.3							
Vancomycin	2010 Horses	0			81.5	14.8	3.7											
	2010 Broilers	0			89.0	8.1	2.9											
	2010 Meat	0			52.9	41.2	5.9											
	2000-07 Broilers	<1			84.4	10.8	4.0	0.3					0.4					
Virginiamycin	2010 Horses	4			70.4	3.7	22.2	3.7										
	2010 Broilers	5		3.7	33.1	33.8	24.3	2.2	2.2	0.7								
	2010 Meat	6		5.9	17.6	41.2	29.4		5.9									
	2000-07 Broilers	17		15.6	34.8	27.9	5.0	10.1	6.1	0.6								

^a MIC in U/mL, see Appendix 3 for details; ^b Data from 2004 and 2007, n=360; ^c Data from 2007, n=197.

Coagulase positive staphylococci from broiler, pig and cattle carcasses

COAGULASE positive staphylococci were isolated from Swedish broiler, pig and beef carcasses in microbial baseline studies performed by the National Food Administration in 2002-2003, 2004-2005 and 2006-2007, respectively.

Materials and Methods

Whole broiler carcasses and swabs from carcasses of pigs and cattle were collected and sent to the National Food Administration for culture. From each animal category, approximately 600 carcasses were sampled at 10-13 of the largest abattoirs, accounting for 90-100% of the Swedish processing of each animal species. In addition, at four low-capacity abattoirs, 150-200 pig carcasses and an equal number of cattle carcasses were sampled. Sampling was random and proportional to slaughter volume.

Broiler carcasses and 400 mL saline were thoroughly mixed. Similarly, swabs from pig and cattle carcasses were mixed with 100 mL saline. Coagulase positive staphylococci were isolated according to procedures by the Nordic Committee on Food Analysis (NMKL nr 66).

Antimicrobial susceptibility was assessed by broth micro-dilution according to CLSI (2008) using VetMIC™ panels. Beta-lactamase production was tested by the nitrocefin test in isolates with MIC to penicillin >1 mg/L and presence of the *mecA* gene by PCR (Smyth et al., 2001) in isolates with MIC to oxacillin >2 mg/L.

Results and comments

Coagulase positive staphylococci were isolated from about two thirds of the broiler carcasses and from one third of the pig and cattle carcasses. In total, 100 broiler isolates, 100 pig isolates and 105 cattle isolates were tested for antimicrobial susceptibility.

Most isolates had MICs to the studied antimicrobials below epidemiological cut-off values (ECOFF) for resistance for *Staphylococcus aureus* (Table). An exception is penicillin where a large proportion of isolates had MICs above 0.12 mg/L which indicate acquired resistance. Also, beta-lactamase production was confirmed in all the 111 isolates tested, i.e. isolates with MICs >1 mg/L. Most likely, all isolates with MICs >0.12 mg/L were beta-lactamase producers.

The distribution of tetracycline MICs for isolates from broiler carcasses appears up-shifted as the expected mode value is 0.5 mg/L and probably a considerable part of the isolates with MICs of 2 mg/L reflect methodological problems. Therefore, the ECOFF of EUCAST has not been applied. However, 13% of the isolates had MICs >64 mg/L which most likely represent true tetracycline resistance.

A small proportion of isolates had MICs above the cut-off value for oxacillin (2 mg/L) indicating possible methicillin resistance (Table). However all these isolates were tested by PCR and the *mecA* gene was not found.

Bacteria contaminating carcasses at slaughter do not necessarily emanate from the animal slaughtered. In Swedish cattle, the prevalence of penicillin resistance in *S. aureus* was 7% in a recent study (Bengtsson et al., 2009). Thus, the high prevalence of penicillin resistance in staphylococci from cattle carcasses indicates other sources for the contamination than cattle.



TABLE H2. Percent resistance (R) and distribution of MICs for coagulase positive staphylococci from carcasses; broilers 2002-03 (n=100), pigs 2004-05 (n=100), cattle 2006-07 (n=105).

Antimicrobial	R (%)	Origin	Distribution (%) of MICs (mg/L)												
			≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Penicillin	16	Broiler		24.0	60.0	2.0		2.0	4.0	6.0	2.0				
	70	Pig		2.0	28.0	10.0	7.0	4.0		49.0					
	63	Cattle	12.4	16.2	8.6	8.6	4.8	3.8	1.9	43.8					
Cefalothin	0	Broiler				16.0	82.0	2.0							
	2	Pig			3.0	46.0	37.0	12.0	1.0	1.0					
	2	Cattle		1.0	11.8	44.1	39.2	2.0	1.0		1.0				
Oxacillin+2% NaCl	7 ^a	Broiler						16.0	77.0	7.0					
	5 ^a	Pig			6.0	6.0	66.0	18.0	5.0	3.0	2.0				
	3 ^a	Cattle			8.6	14.3	43.8	24.8	5.7		2.9				
Erythromycin	5	Broiler					59.0	36.0	1.0		4.0				
	6	Pig					53.0	41.0				2.0	4.0		
	3	Cattle				11.4	55.2	30.5				1.0	1.9		
Chloramphenicol	0	Broiler								2.0	86.0	12.0			
	1	Pig								4.0	76.0	19.0	1.0		
	1	Cattle					1.0			31.4	63.8	2.9	1.0		
Tetracycline	(13) ^b	Broiler					43.0	34.0	11.0					13.0	
	3	Pig					55.0	42.0	2.0					1.0	
	5	Cattle					93.3	1.9	1.0		1.0		1.9	1.0	
Fusidic acid	3	Pig			6.0	38.0	53.0	1.0	1.0	1.0					
	3	Cattle		1.0	15.2	52.4	28.6			1.9	1.0				
Gentamicin	0	Broiler				26.0	63.0	9.0	2.0						
	2	Pig					40.0	46.0	12.0	2.0					
	1	Cattle					59.0	35.2	4.8		1.0				
Kanamycin	4	Pig						2.0	36.0	46.0	12.0	4.0			
	2	Cattle				1.9	3.8	26.7	55.2	9.5	1.0		1.9		
Ciprofloxacin	0	Pig			10.0	68.0	21.0	1.0							
	2	Cattle		1.9	32.4	50.5	12.4	1.0	1.0	1.0					

^a *mecA* gene not found. ^b Due to methodological uncertainties, the EUCAST ECCOF is not applied. See comments in text.

Animal pathogens

ISOLATES TESTED are from clinical submissions of samples to SVA if not otherwise stated. For these samples, information on the indications for sampling is not available but the vast majority of submissions are likely from diseased animals. Therefore, data are probably biased towards samples from treated animals or from herds where antimicrobial treatments are common. Any assessment of trends is based on the assumption that this bias is inherent throughout the observation period.

In SVARM, isolates are, when possible, classified as susceptible or resistant by epidemiological cut-off values issued by EUCAST (see Guidance for readers and Appendix 3 for details). This classifies isolates with acquired reduced susceptibility as resistant, which is relevant for monitoring purposes, but it should be understood that this not always implies clinical resistance.

Pig

Escherichia coli

Isolates of *Escherichia coli* from years 1992–2010 are from clinical submissions of samples from the gastro-intestinal tract (intestinal content, faecal samples or mesenteric lymph nodes), while data from 1989–1991 include all *E. coli* isolated from pigs, irrespective of material type.

Before the first of October 2007, all *E. coli* isolated from the gastro-intestinal tract were susceptibility tested. After that date, the criteria for susceptibility testing were changed and only *E. coli* that harbour genes coding for virulence factors are tested for susceptibility. The following genes are analysed by PCR: enterotoxin (LT), heat-stable enterotoxin a and b (STa and STb), verocytotoxin (VT2e) and adhesions factors F4, F5, F6, F18 and F41. Isolates with at least one of these genes were susceptibility tested.

As in previous years, resistance to ampicillin, streptomycin, tetracycline or trimethoprim-sulphonamides in *E. coli* was most commonly occurring in 2010 (Table Pig I). In the 70s and 80s, prevalence of *E. coli* resistant to ampicillin was around seven percent (Franklin, 1976; Franklin, 1984). From the late 90s to year 2004, prevalence of ampicillin resistance rose gradually to 22%. Since then, the figures seem to have stabilised around 20%. In 2010, all of the ampicillin resistant isolates were resistant to at least one other antimicrobial. Multiresistance occurred in 15% of the isolates. This figure was 19% in 2009 and 14% in 2008. The resistance combination ampicillin, streptomycin and trimethoprim-sulphonamides was the most common trait, occurring in 71% of the multiresistant isolates. Four percent of the isolates were resistant to four or more antimicrobials and two percent were resistant to five or more.

Brachyspira hyodysenteriae

Isolates of *Brachyspira hyodysenteriae* are from clinical submissions of faecal samples from pigs. All isolates were susceptible to tiamulin (Table Pig II). In the late 80s, susceptibility of *B. hyodysenteriae* was tested with an agar dilution technique, and 20% of the isolates were resistant to tylosin (Gunnarsson et al., 1991). In year 2001, the figure had increased dramatically to around 80% (Table Pig II).

The last three years isolates were susceptibility tested also for tylvalosin, a macrolide authorised for treatment of swine dysentery in the European Union. No cut-off value for resistance to tylvalosin is available and due to the small number of *B. hyodysenteriae* tested a value cannot be determined from the distribution of MICs. However, Karlsson et al. (2004) showed a correlation between the MICs of tylosin and tylvalosin indicating that macrolide resistance caused by structural changes of ribosomal RNA also affects the binding of tylvalosin. Since

TABLE FIG I. Resistance (%) in *Escherichia coli* from pigs 1989–2010 and distribution of MICs for isolates from 2010. Isolates are from clinical submissions of faecal samples or samples taken post mortem from the gastro-intestinal tract.

Antimicrobial	Resistance (%)								Distribution (%) of MICs (mg/L)									
	1989-91 n=248	1992-94 n=431	1995-97 n=1244	1998-00 n=1074	2001-03 n=935	2004-06 n=1009	2007-09 n=278	2010 n=94	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	6	10	9	11	17	22	21	20				1.1	41.5	37.2				20.2
Ceftiofur	-	-	-	-	<1 ^g	<1	0	0		71.3	23.4	5.3						
Enrofloxacin ^a	1 ^f	7	5	6	8	9	7	6	93.6	2.1	3.2	1.1						
Florfenicol	-	-	-	-	<1 ^g	<1	0	0					10.6	62.8	24.5	2.1		
Gentamicin ^b	1	1	<1	1	4	1	<1	0					97.9	2.1				
Neomycin	17	14	9	6	5	4	6	1						97.9	1.1		1.1	
Streptomycin ^c	44	44	32	30	36	36	35	28						21.3	35.1	16.0	8.5	19.1
Tetracycline	28	35	31	33	30	26	26	21				37.2	29.8	9.6	2.1			21.3
Trim/Sulph. ^{d,e}	17	15	13	14	19	25	20	23		71.3	5.3							23.4

^a Cut-off value >0.25 mg/L until 2001; ^b Cut-off value >8 mg/L until 2002; ^c Cut-off value >32 mg/L until 2001; ^d Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); ^e Cut-off value >4 mg/L until year 2001; ^f 227 isolates tested; ^g 688 isolates tested.

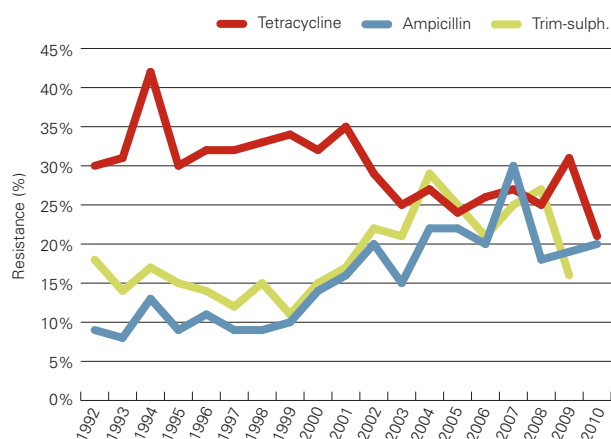


FIGURE PIG. Resistance (%) to ampicillin, tetracycline and trimethoprim-sulphamethoxazole in *Escherichia coli* from pigs 1992-2010.

2005 isolates have been susceptibility tested for doxycycline and valnemulin. Cut-off values are not available for these substances either.

Sweden has a programme for controlling swine dysentery by three strategies; testing of nucleus and multiplying herds for *B. hyodysenteriae* twice a year, eradication of the bacteria in infected herds and tracing the source of infection. Nevertheless, it is imperative that all herds where treatment failure is suspected are thoroughly investigated. Since only macrolides and tiamulin are authorised for treatment of swine dysentery in pigs it is important to monitor resistance development in *B. hyodysenteriae*. The number of isolates available for susceptibility testing has decreased during the year and compared to 2009 the number of samples taken has decreased.

A possible explanation for that is a successful reduction of swine dysentery. There is also a decline in sales figures for tiamulin (see “Use of antimicrobials”)

Brachyspira pilosicoli

Isolates of *Brachyspira pilosicoli* are from clinical submissions of faecal samples from pigs. In 2001, the first isolates of *B. pilosicoli* resistant to tiamulin were confirmed in Sweden. These isolates were associated with treatment failure in a Swedish pig herd with spirochaetal diarrhoea (see SVARM 2003). Since then, tiamulin resistant strains have been isolated every year but there is no apparent increasing trend in prevalence of resistance (Table Pig III). The frequency of resistance to tylosin has been around 60% during the last years (Table Pig III).

Tylvalosin is a macrolide authorised for treatment of swine dysentery in the European Union, although, not for treatment of spirochaetal diarrhoea. Twenty-one of twenty-six isolates from 2008-2010 with MICs >128 mg/L of tylosin have MICs of tylvalosin of 32 or more. A correlation between MICs of tylosin and tylvalosin was shown by Karlsson et al. (2004) for *B. hyodysenteriae*. This indicates that macrolide resistance caused by structural changes of ribosomal RNA also affects the binding of tylvalosin. With this background, together with the distribution of the MICs in this material a cut-off value for tylvalosin of >4 mg/L is suggested.

During 2008 and 2009, four isolates were resistant to tiamulin, tylosin and tylvalosin. Although such isolates may be susceptible to other antimicrobials, only tiamulin and tylosin are currently licensed for treatment of spirochaetal diarrhoea in pigs in Sweden. Susceptibility testing of *B. pilosicoli* from herds where tiamulin is to be used is of importance.

TABLE PIG II. Resistance (%) in *Brachyspira hyodysenteriae* from pigs 2001-2003 and 2005-2010 and distribution of MICs for isolates from 2005-2010. Isolates are from clinical submissions of faecal samples.

Antimicrobial	Resistance (%)				Distribution (%) of MICs (mg/L)													
	2001 n=75	2002 n=109	2003 n=100	2005-10 n=125	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Doxycycline	-	-	-	ND ^a			16.8	64.0	6.4	7.2	5.6							
Tiamulin	0	0	0	0		28.8	48.0	12.0	8.8	2.4								
Tylosin	83	73	89	77						0.8	10.4	11.2	0.8				2.4	74.4
Tylvalosin	-	-	-	ND ^{a,b}					2.1	22.9	4.2	18.8	27.1	16.7	2.1	4.2		
Valnemulin	-	-	-	ND ^a	78.4	12.8	2.4	4.0	2.4									

^a ND=not determined because no cut-off value is available; ^b48 isolates tested.

TABLE PIG III. Resistance (%) in *Brachyspira pilosicoli* from pigs 2002-2003 and 2005-2010 and distribution of MICs for isolates from 2005-2010. Isolates are from clinical submissions of faecal samples.

Antimicrobial	Resistance (%)				Distribution (%) of MICs (mg/L)											
	2002-03 n=93	2005-10 n=231	≤0.03	≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Doxycycline	-	ND ^b			39.8	47.6	3.9	3.0	5.2	0.4						
Tiamulin	14	12		29.0	28.1	13.0	8.7	6.9	1.7		2.2	10.0				
Tylosin	50 ^a	62							4.8	18.6	11.3	3.9	4.3	3.5	5.2	48.5
Tylvalosin	-	38 ^c					7.4	14.7	32.4	7.4	2.9	2.9	17.6	14.7		
Valnemulin	-	ND ^b	42.9	21.6	6.5	9.1	6.5	5.2	2.2	1.7	4.3					

^a 86 isolates tested; ^b ND=not determined because no cut-off value is available; ^c 68 isolates tested.

TABLE PIG IV. Resistance (%) in *Actinobacillus pleuropneumoniae* from pigs 1992-2000 and 2005-2010. Distribution of MICs for isolates from 2008-2010. Isolates are from clinical submissions of samples from the respiratory tract or from post mortem investigations of lungs.

Antimicrobial	Resistance (%)			Distribution (%) of MICs (mg/L)														
	1992-00 n=18	2005-07 n=84	2008-10 n=79	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Ampicillin	6	0	0			20.3	59.5	20.3										
Cefotaxime	-	0	0		100.0													
Ceftiofur	-	0	0 ^c			100.0												
Chloramph.	11	0	0							100.0								
Ciprofloxacin	6 ^a	0	0	69.6	30.4													
Florfenicol	-	0	0								100.0							
Gentamicin	-	0	0						1.3	6.3	72.2	20.3						
Nalidixic acid	-	0	0						2.5	50.6	46.8							
Penicillin	6	0	0			1.3	65.8	32.9										
Streptomycin	-	0	1									1.3	45.6	51.9	1.3			
Tetracycline	11 ^b	1	0						100.0									
Trimethoprim	-	0	0				77.2	15.2	6.3	1.3								

^a Enrofloxacin tested, cut-off value 2 mg/L.; ^b cut-off value >8 mg/L.; ^c 63 isolates tested.

TABLE PIG V. Resistance (%) in *Pasteurella* spp. from pigs 2000-2001 and 2005-2010. Distribution of MICs for isolates from 2008-2010. Isolates are from the respiratory tract, isolated from nasal swabs or from post mortem investigations of lungs.

Antimicrobial	Resistance (%)			Distribution (%) of MICs (mg/L)																
	2000-01 n=75	2005-07 n=38	2008-10 n=59	≤0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Ampicillin	0	0	0							100.0										
Cefotaxime	-	0	0				100.0													
Chloramph.	1	0	0									100.0								
Ciprofloxacin	1 ^a	0	0	11.9	61.0	25.4	1.7													
Florfenicol	-	0	0										100.0							
Gentamicin	4	0	0							3.4	74.6	22.0								
Nalidixic acid	-	0	0							42.4	44.1	11.9		1.7						
Penicillin	0	0	0				1.7	13.6	69.5	15.3										
Streptomycin	4	0	2										3.4	40.7	37.3	16.9	1.7			
Tetracycline	1	0	0							98.3	1.7									
Trimethoprim	-	0	0					69.5	28.8	1.7										

^a Enrofloxacin tested, cut-off value 2 mg/L.

Actinobacillus pleuropneumoniae

Isolates of *Actinobacillus pleuropneumoniae* from years 1992-2000 are from the respiratory tract (nasal swabs and lung, including regional lymph nodes) but from years 2005-2010 all isolates are from lungs sampled post mortem.

Since 2005, *A. pleuropneumoniae* has been susceptible to almost all antimicrobials tested (Table Pig IV). Pneumonia caused by *A. pleuropneumoniae* is an important disease in Swedish pig production and a higher frequency of sampling and susceptibility testing is desirable if emerging resistance is to be detected early. Before 2005 only sporadic isolates of *A. pleuropneumoniae* were susceptibility tested. In 2005, the surveillance programme SVARMpat for antimicrobial resist-

ance started and although the number of strains tested yearly has increased it is still low.

Pasteurella spp.

Isolates of *Pasteurella* spp. are from nasal swabs collected within a control programme for atrophic rhinitis in nucleus and multiplying herds or from post mortem investigation of lungs. Isolates from the control programme are likely from healthy pigs, whereas isolates from post mortem investigations of lungs are most likely from pigs with respiratory problems. Since 2005, *Pasteurella* spp has been susceptible to most antimicrobials tested (Table Pig V).

Cattle

Escherichia coli

Isolates of *Escherichia coli* are from the gastro-intestinal tract of calves. The frequency of resistance to ampicillin, neomycin, streptomycin and tetracycline was above 20% in 2007–2010 (Table Cattle I). Tetracycline was the most common trait occurring in more than half of the isolates. Multiresistance occurred in 21 isolates (38%). This is higher than previous years but the small number of isolates tested precludes conclusions on trends. The resistance combination ampicillin, streptomycin and tetracycline was the most common combination, occurring in 22% of the isolates. Two isolates from 2010 had MICs (2 mg/L) above the cut-off value for ceftiofur. These two isolates were not available for further investigation. Judging by the MIC, the results are probably due to methodological error, or the isolates express chromosomal AmpC.

Pasteurella spp.

Isolates of *Pasteurella* spp. from 2005 to 2009 are from clinical submissions of samples from calves with respiratory disease or from post-mortem investigations of lungs. The isolates from years 1997–2000 are from a field study on respiratory pathogens in calves presented in SVARM 2000.

Antimicrobial resistance among isolates of *Pasteurella* spp. is rare (Table Cattle II). One isolate in 2009 and one in 2010 had MICs above the cut-off value for ceftiofur. This is most likely not a true value, since the MICs for penicillin was 0.12 mg/L and 0.25 mg/L, respectively. Isolates of beta-lactamase producing *Pasteurella* spp. were confirmed in Sweden from one herd in 2003. Since 2005, resistance to penicillin and tetracycline, the substances commonly used for therapy of respiratory disease in calves, has not been detected in *Pasteurella* spp. However, in 2010 an isolate of beta-lactamase producing *Mannheimia haemolytica* from a calf with pneumonia was confirmed after post mortem investigation of the lungs. However, the isolate was susceptible for tetracycline, quinolones and cefotaxime. The herd was known to have respiratory problems and a few months later an isolate of *Mannheimia haemolytica* with the same resistance pattern was isolated from another calf, indicating that this strain persisted in the herd.

Penicillin is considered the substance of choice for treatment of pneumonia in calves. The number of isolates tested is low and more frequent sampling of calves with respiratory disorders and subsequent susceptibility testing is desirable if emerging resistance is to be detected early.

TABLE CATTLE I. Occurrence of resistance among *Escherichia coli* from cattle 1992–2002, 2004 and 2005–2010. Distribution of MICs for isolates from 2007–2010. Isolates are from diagnostic submissions of faecal samples or samples taken post mortem from the gastro-intestinal tract, except isolates from 2004 which are from a study of both healthy and diseased calves.

Antimicrobial	Resistance (%)				Distribution (%) of MICs (mg/L)									
	1992-02 n=220	2004 n=87 ^h	2005-06 n=63	2007-10 n=55	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	24	29	32	31				1.8	45.5	21.8		30.9		
Ceftiofur ^a	0 ^g	0	0	4		29.1	61.8	5.5	3.6					
Enrofloxacin ^b	10	14	13	7	92.7	1.8	1.8		3.6					
Florfenicol	0 ^g	0	0	2					5.5	27.3	63.6	1.8	1.8	
Gentamicin ^c	5	0	0	2					80.0	18.2			1.8	
Neomycin	8	7	13	22						70.9	7.3		1.8	20.0
Streptomycin ^d	42	48	54	49						1.8	23.6	25.5		49.1
Tetracycline	31	37	49	58				16.4	16.4	7.3	1.8	58.2		
Trim/Sulph. ^{e,f}	11	10	21	18			81.8				18.2			

^a Cut-off value >2 mg/L until 2006; ^b Cut-off value >0.25 mg/L until 2004; ^c Cut-off value >8 mg/L until 2001; ^d Cut-off value >32 mg/L until 2006; ^e Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); ^f Cut-off value >4 mg/L until 2006; ^g 16 isolates tested; ^h 1/3 of the isolates were from calves with diarrhoea.

TABLE CATTLE II. Resistance (%) in *Pasteurella* spp. from calves 1997–2000 and 2005–2010. Distribution of MICs for isolates from 2008–2010. Isolates are from the respiratory tract, isolated from nasal swabs or from post mortem investigations of lungs.

Antimicrobial	Resistance (%)			Distribution (%) of MICs (mg/L)									
	1997-00 n=254	2005-07 n=27	2008-10 n=71	≤0.06	0.12	0.25	0.5	1	2	4	8	16	>16
Ampicillin	1	0	0					100.0					
Ceftiofur	-	0	3 ^b			97.0	1.5	1.5					
Enrofloxacin	2	0	0 ^c		100.0								
Florfenicol	-	0	0						91.5	8.5			
Penicillin	0	0	0		36.7	54.9	8.5						
Tetracycline	3	0	0					98.6	1.4				
Trim/Sulph. ^a	2	0	0				98.6		1.4				

^a Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); ^b 65 isolates tested; ^c 46 isolates tested.

Farmed fish

Isolates of *Aeromonas salmonicida* subsp. *achromogenes*, *Flavobacter columnare* and *Flavobacter psychrophilum* are from clinical submissions of farmed fish. Most isolates represent a unique batch of fish but occasional isolates are duplicates within the same batch. Antimicrobial susceptibility was tested by micro-dilution according recommendations by Alderman & Smith (2001). At SVA this methodology is used for routine testing of isolates from clinical submissions of fish.

This year data for 8 isolates of *A. salmonicida* subsp. *achromogenes*, 5 of *F. columnare* and 21 of *F. psychrophilum* were available. The majority of the two former bacterial species are from brown trout whereas most of *F. psychrophilum* are from rainbow trout. A similar distribution among fish species applies

for isolates from previous years. Data for 2010 and 2009 are compiled and presented as distributions of MICs in Table Fish I.

At present there are no accepted interpretative criteria for MIC data of bacteria from aquaculture. But evaluation of the distributions of MICs indicates the presence of isolates with reduced susceptibility, i.e. deviating high MICs, (Table Fish I). For example, MIC distributions for the quinolone nalidixic acid are bimodal in all three bacterial species. This indicates the presence of acquired resistance to quinolones. Likewise deviating high MICs for tetracycline in *Flavobacter*, and for florfenicol among *A. salmonicida* and *F. columnare*, indicate acquired resistance to these antimicrobials. Resistance to these antimicrobials is reasonable since there is a limited therapeutic use of the quinolone oxolinic acid as well as of tetracycline and florfenicol in aquaculture in Sweden.

TABLE FISH I. Distribution of MICs for *Aeromonas salmonicida* subsp. *achromogenes*, *Flavobacter columnare* and *Flavobacter psychrophilum* from farmed fish, 2005-2010.

Bacterial species	Antimicrobial	Year	Number of isolates	Distribution (%) of MICs (mg/L)									
				≤0.25	0.5	1	2	4	8	16	32	64	>64
<i>Aeromonas salmonicida</i> subsp. <i>achromogenes</i>	Florfenicol	2009-10	31				96.8			3.2			
		2005-08	87				96.6	2.3	1.1				
	Nalidixic acid	2009-10	31		80.6	3.2						9.7	6.5
		2005-08	87		80.5	4.6				1.1	3.4	5.7	4.6
	Tetracycline	2009-10	31	80.6	12.9	3.2				3.2			
		2005-08	87	90.8	8.0				1.1				
<i>Flavobacter columnare</i>	Florfenicol	2009-10	15				100.0						
		2005-08	46				95.7	2.2			2.2		
	Nalidixic acid	2009-10	15		80.0	6.7	6.7	6.7					
		2005-08	46		73.9	13.0	4.3				2.2	2.2	4.3
	Tetracycline	2009-10	15	73.3	26.7								
		2005-08	46	84.8	6.5	4.3			2.2		2.2		
<i>Flavobacter psychrophilum</i>	Florfenicol	2009-10	45				97.8		2.2				
		2005-08	69				98.6	1.4					
	Nalidixic acid	2009-10	45				17.8	26.7	2.2	2.2	6.7	8.9	35.6
		2005-08	69		7.2		37.7	39.1		1.4	1.4		13.0
	Tetracycline	2009-10	45	40.0	4.4	22.2	6.7	22.2	4.4				
		2005-08	69	72.5	5.8	5.8	7.2	5.8	1.4	1.4			

SVARMpat

THE PURPOSE of SVARMpat is to increase the knowledge on resistance in animal pathogens from farm animals and the program has been running since 2005 (for more information See SVARM 2005). SVARMpat is a co-operation between the National Veterinary Institute (SVA) and the Swedish Animal Health Service and is financed by the Swedish Board of Agriculture. Results are reported yearly in the SVARM report, in newsletters directly to veterinary practitioners, in articles in popular science and in scientific publications. The purpose is to inform practitioners and animal owners about activities and results, but also to deepen their knowledge on antimicrobials, antimicrobial treatment and resistance.

An important activity in SVARMpat is to encourage practitioners and pathologists to submit samples for microbiological culture and susceptibility testing. In the SVARM report some results from this work are presented, e.g. susceptibility data on *Actinobacillus pleuropneumoniae* and *Brachyspira* spp. from pigs, *Pasteurella* spp. from both cattle and pigs and *Escherichia coli* from calves.

Activities in SVARMpat 2010:

- **Screening for MRSA in milk samples** from cows was started and will continue during 2011. Isolates of beta-lactamase producing *Staphylococcus aureus* are investigated for methicillin resistance. Since the identity of the samples is not known, they cannot be traced to herd. During 2010, 206 isolates were investigated and none of them were confirmed as MRSA.
- ***Mycoplasma bovis* in calves with pneumonia.** Nasal swabs were taken from calves with respiratory symptoms. Samples were also taken at post mortem investigation of lungs from calves with pneumonia. During 2010, samples from 85 calves were investigated and *Mycoplasma bovis* was not found. *M. bovis* as the causative agent of pneumonia in calves would have implications for the antibiotic treatment regime. Since *M. bovis* not have been found, so far, there is still no bacteriological reason for treatment with other substances than penicillin.
- ***Mycoplasma ovipneumoniae* in sheep with pneumonia.** Samples were taken at post mortem investigation of lungs from sheep with pneumonia. During 2010, samples from 15 sheep were analysed and *M. ovipneumoniae* was found in five samples.
- **Sampling from cattle with interdigital necrobacillosis** and susceptibility testing of *Fusobacterium necrophorum* continued during 2010. Focus during 2010 was also on the significance of sampling technique for isolation of *F. necrophorum* and on a questionnaire about treatment regimes and experiences of treatment failure. Resistance to penicillin was not detected in the study, supporting the opinion that penicillin is the substance of choice for treatment of interdigital necrobacillosis.
- **Investigation of causative pathogens in arthritis in suckling piglets** and the antimicrobial susceptibility of these bacteria. One lame piglet per herd with more than 100 sows was euthanized and an autopsy was performed together with bacteriological sampling of two joints. During 2010, 93 piglets were analysed. *Streptococcus equisimilis* dominated the bacteriological findings, followed by *Staphylococcus hyicus* and *E. coli*. All streptococci, but less than half of the staphylococci were susceptible to penicillin. The study will be completed during 2011.
- **Screening for MRSA in slaughter pigs.** Nasal swabs were taken at slaughter from 191 herds, five pigs per herd. Samples from the same herd were pooled at the laboratory. One of the 191 samples was positive. This was the first finding of MRSA in production animals in Sweden. (See "Zoonotic bacteria".)
- **The PhD project "Vancomycin resistant enterococci in Swedish broilers"** is partly financed by SVARMpat. In the project, the spread of vancomycin resistant enterococci (VRE) in Swedish broilers since 2000 is investigated. The aim is to elucidate the epidemiology of VRE in broilers and, if possible, to mitigate further spread and reduce the prevalence on farms where VRE already occur. See also SVARM 2008 for details. The project started in 2007 and will be completed during 2011.

Horse

Escherichia coli

The isolates of *Escherichia coli* included are from the genital tract of mares. As in previous years, resistance to trimethoprim-sulphonamides or streptomycin are the most common resistance traits (Table Horse I). Trimethoprim-sulphonamide resistance is probably a consequence of the frequent use of this antimicrobial combination in horses. Since the introduction of trimethoprim-sulphonamides on the Swedish market as an oral formulation for horses in the late 80s, the prevalence of resistance in *E. coli* has increased from 2% in years 1992-1994. However, this year's figure is the lowest since the beginning of this decade and in addition use of the combination trimethoprim-sulphonamides has decreased by 20% since 2006 ("Use of antimicrobials, Horses").

Multiresistance occurred in 4% of the isolates. Two-thirds of multiresistant *E. coli* were resistant to ampicillin, gentamicin and trimethoprim-sulphonamides. None of the multiresistant isolates were resistant to five or more substances.

Resistance to ceftiofur only occurred in one isolate. This isolate was tested for production of extended-spectrum beta-lactamases (ESBL) according to CLSI (see Appendix 3) and

was genotypically confirmed to produce CTX-M-1. Although resistance to 3rd generation cephalosporins is rare in this year's material, a majority of the ESBL producing *E. coli* in Sweden isolated from diagnostic submissions are from the genital tract of mares (see Highlight "Enterobacteriaceae producing extended spectrum beta-lactamases (ESBL) – isolates from diagnostic submissions"). Close monitoring of the situation is therefore strongly recommended.

Streptococcus zooepidemicus

The isolates included are from the respiratory tract of horses. As in previous years, *Streptococcus zooepidemicus* are uniformly susceptible to penicillin (Table Horse II). Occurrence of resistance to trimethoprim-sulphonamides has been high during the last 15 years, probably due to the common use of oral trimethoprim-sulphonamides in horses, but this year the occurrence has decreased to 7%. However, the number of analysed isolates are fewer in 2010 compared to earlier years and data should be interpreted with caution. *Streptococcus zooepidemicus* has a low inherent susceptibility to fluoroquinolones and aminoglycosides (i.e. gentamicin) and it can be observed that MICs are above concentrations that can be obtained during systemic therapy with these antimicrobials.

TABLE HORSE I. Resistance (%) in *Escherichia coli* from horses 1992-2010 and distribution of MICs for isolates from 2010. Isolates are from clinical submissions of samples from the female genital tract.

Antimicrobial	Resistance (%)							Distribution (%) of MICs (mg/L)									
	1992-94 n=48	1995-97 n=216	1998-00 n=222	2001-03 n=457	2004-06 n=473	2007-09 n=657	2010 n=236	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	15	17	10	9	7	10	7					19.0	63.6	10.2	7.2		
Ceftiofur	-	-	-	<1	<1	2	<1		43.2	51.3	5.1		0.4				
Enrofloxacin ^a	8	3	3	2	4	2	5	94.9	1.7	0.4	1.3	1.7					
Florfenicol	-	-	-	0	0	<1	<1					5.1	56.4	36.9	1.3	0.4	
Gentamicin ^b	0	3	6	6	2	4	2					94.9	2.5			2.5	
Neomycin	4	5	5	3	4	2	1						95.3	3.4		0.4	0.8
Streptomycin ^c	31	24	21	23	21	21	15						5.5	53.0	26.3	3.4	11.9
Tetracycline	6	5	9	6	8	7	5				21.6	67.8	5.1	0.8	4.7		
Trim/Sulph. ^d	2	15	17	18	17	20	13			85.2	1.7	0.8	0.4	11.9			

^a Cut-off value >0.25 mg/L until 2002; ^b Cut-off value >8 mg/L until 2002; ^c Cut-off value >16 mg/L until 2001; ^d Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole) and cut-off value >4 mg/L until 2001.

TABLE HORSE II. Resistance (%) in *Streptococcus zooepidemicus* from horses 1992-2010 and distribution of MICs for isolates from 2010. Isolates are from clinical submissions of samples from the respiratory tract.

Antimicrobial	Resistance (%)							Distribution (%) of MICs (mg/L)									
	1992-94 n=218	1995-97 n=402	1998-00 n=409	2001-03 n=505	2004-06 n=534	2007-09 n=491	2010 n=43	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	0	<1	0	0	0	0	0				100						
Enrofloxacin	NR ^b	NR	NR	NR	NR	NR	NR			2.3	32.6	65.1					
Florfenicol	-	-	-	1	<1	0	0					93.0	7.0				
Gentamicin	NR	NR	NR	NR	NR	NR	NR							30.2	65.1	4.6	
Penicillin	0	<1	0	0	0	0	0	100									
Spiramycin	<1	1	0	1	<1	<1	0						100				
Tetracycline	4	3	4	5	3	3	7				32.6	58.1	2.3		7.0		
Trim/Sulph. ^a	1	11	57	36	42	18	7			76.7	11.6	4.6		7.0			

^a Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); ^b NR= Not relevant as the inherent susceptibility is above concentrations that can be obtained during therapy.

Staphylococcus aureus

Isolates of *Staphylococcus aureus* are from skin samples, excluding wounds and abscesses. The number of resistant *S. aureus* has been stable during the last three years and resistance to penicillin is dominating (Table Horse III). In 2010, 21% of the isolates were resistant to penicillin due to penicillinase production. Still, penicillin should be considered the drug of choice if antimicrobial treatment is necessary in horses with skin disorders. Investigations on the underlying causes are of course crucial.

One isolate had MIC >1 mg/L for oxacillin and was tested by PCR for the presence of *mecA*-gene and was positive, i.e. methicillin resistant. More information on methicillin resistant *S. aureus* (MRSA) isolated from horses in Sweden is presented in the chapter "Zoonotic bacteria".

Occurrence of multiresistance (i.e. resistance to three or more antimicrobials) was rare. Only one isolate was multiresistant (0.8%); to enrofloxacin, gentamicin and tetracycline. No isolate was resistant to more than three substances.

TABLE HORSE III. Resistance (%) in *Staphylococcus aureus* from horses 2007-2010 and distribution of MICs for isolates from 2010. Isolates are from clinical submissions of samples from skin.

Antimicrobial	Resistance (%)				Distribution (%) of MICs (mg/L)									
	2007 n=113	2008 n=99	2009 n=96	2010 n=131	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ceftiofur	0	2	2	<1		1.5	16.0	80.2	1.5	0.8				
Enrofloxacin	3	2	2	2	34.4	59.5	3.8	2.3						
Florfenicol	2	3	1	<1					6.9	74.8	17.6	0.8		
Gentamicin	9	7	6	7					93.1	3.0	0.8		3.1	
Oxacillin	-	-	2	<1			96.9	2.3	0.8					
Penicillin ^a	26	36	36	21										
Spiramycin	1	0	0	0						32.1	58.0	9.2		
Streptomycin	12	14	9	5						55.0	27.5	12.2	0.8	4.6
Tetracycline	2	6	4	<1				96.9	0.8					
Trim/Sulph. ^b	4	5	3	2			97.7	1.5	0.8					

^a Denotes beta-lactamase production; ^b Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole).



Enterobacteriaceae producing extended spectrum beta-lactamases (ESBL) – isolates from diagnostic submissions

THIS HIGHLIGHT summarises information on bacterial isolates producing extended spectrum beta-lactamases (ESBL) from diagnostic samples. The isolates were sent to the Section of Antibiotics at the National Veterinary Institute (SVA) because of resistance to 3rd generation cephalosporins.

During 2008 to 2011, 28 isolates (16 *Escherichia coli*, 7 *Enterobacter* spp., 3 *Klebsiella pneumoniae*, 1 *Klebsiella oxytoca*, and 1 *Escherichia hermannii*) were phenotypically ESBL producing and a majority of these were isolated from horses (68%). Of the 19 isolates from horses, seven originated from the genital tract in mares. The number of ESBL producing bacteria per year and animal species are shown in the table below. Genotypic tests were done on 22 isolates and the beta-lactamases found belonged either to group CTX-M-1 or SHV of ESBL-type.

The ESBL producing isolates from horses were also resistant to gentamicin, trimethoprim and sulphonamides. More than half (63%) of the isolates were also resistant to tetracycline. However, they were susceptible to fluoroquinolones and florfenicol. Resistance in ESBL producing isolates from dogs and cats varied.

During 2010, 200 diagnostic submissions from the female reproductive tract in horses were screened for ESBL producing bacteria by culture on selective media, i.e. MacConkey agar with cefotaxime added (1 mg/L). From eight samples, *Klebsiella pneumoniae* that produced SHV of ESBL type were isolated and half of them originated from the same stud farm. In addition, 431 faecal samples from horses at stud farms and on arrival to animal hospitals were cultured on the selective media. Of these samples, 147 were taken from mares at stud farms and 284 were collected from horses consecutively on arrival to five hospitals. Six faecal samples contained *Escherichia coli* producing SHV of ESBL-type.

In conclusion, ESBL producing *Enterobacteriaceae* are still uncommon in routine clinical diagnostic samples but the situation in horses warrants closer monitoring. Routine diagnostic laboratories are advised to submit isolates of *Enterobacteriaceae* phenotypically resistant to 3rd generation cephalosporins to the Section of Antibiotics at SVA, where confirmatory phenotypic and genotypic tests for ESBL and AmpC are performed (See Appendix 3).

In a clinical condition where the patient needs to be treated with antimicrobials, multiresistant *Enterobacteriaceae* pose a challenge for the veterinarian, especially in horses, since the number of antimicrobials licensed is limited. Increased awareness of the need for infection control and antimicrobial stewardship is essential to minimize the spread of these resistant bacteria.

TABLE. Number of confirmed extended spectrum beta-lactamases (ESBL) producing *Enterobacteriaceae* isolated from diagnostic submissions from animals during 2008-2010.

Animal species	Bacterial species	2008	2009	2010
Cat	<i>Enterobacter</i> spp.	-	-	-
	<i>Escherichia coli</i>	-	-	2
	<i>Klebsiella oxytoca</i>	-	-	1
Dog	<i>Enterobacter</i> spp.	-	1	2
	<i>Escherichia coli</i>	1	-	1
	<i>Klebsiella pneumoniae</i>	-	1	-
Horse	<i>Enterobacter</i> spp.	-	1	3
	<i>Escherichia coli</i>	2	3	7
	<i>Klebsiella pneumoniae</i>	-	1	1
	<i>Escherichia hermannii</i>	-	-	1

Dog

Escherichia coli

Isolates of *Escherichia coli* are from samples of urine, submitted either as urine or as dip-slide cultures. Resistance to ampicillin was the most common resistance trait.

The isolates were tested for susceptibility to cefotaxime as an indicator of extended-spectrum beta-lactamase (ESBL) production and not as a treatment option. The six isolates with MIC for cefotaxime >1 mg/L were further tested and all were confirmed as AmpC producing. Further analyses are needed to clarify whether the AmpC gene is chromosomal or placed on a plasmid. For more information on resistance to 3rd generation cephalosporins see highlight “*Enterobacteriaceae* producing extended spectrum beta-lactamases (ESBL) – isolates from diagnostic submissions”.

Multiresistance occurred in 5% of the isolates and this figure is on the same level as last year. Of the multiresistant isolates, 60% were resistant to at least ampicillin, trimethoprim-sulphonamides and tetracycline. Only four *E. coli* isolates were resistant to five or more antimicrobials i.e. <1% of all isolates.

Uncomplicated cystitis in dogs is frequently treated with aminopenicillins, which are by far the most commonly prescribed antimicrobials for dogs (see “Use of antimicrobials”). However, from the resistance data presented, an even better first choice for treatment of uncomplicated cystitis would be nitrofurantoin or trimethoprim-sulphonamides. However, none of these substances are authorised for use in dogs.

Staphylococcus pseudintermedius

Staphylococcus pseudintermedius included are from skin samples. In 2005, *S. pseudintermedius*, a novel staphylococcal species was described (Devriese et al., 2005). Further on Sasaki et al. (2007) and Bannoehr et al. (2007) reported that canine strains of *S. intermedius* should be classified as *S. pseudintermedius*. Therefore, it was proposed to report strains from dogs as *S. pseudintermedius*, unless genomic investigations prove that the strain belongs to another related species (Devriese et al., 2009). Consequently, resistance data on *S. intermedius* from previous SVARM reports should be regarded as resistance data on *S. pseudintermedius*.

As in previous years, the prevalence of resistance to penicillin due to production of beta-lactamases (penicillinase) in *S. pseudintermedius* is high, 86% (Table Dog II). Already in the late 70s, 70% of *S. pseudintermedius* were resistant to penicillin (Franklin, 1978) and during the last two decades, the resistance frequency has been around 80-90%. Besides penicillin, resistance to clindamycin, erythromycin, fusidic acid or tetracycline was common in 2010, as in previous years. In 2008, resistance to clindamycin was 22%, increased to 27% in 2009 and in this year's material 26% of the isolates were resistant to clindamycin. This could be an increase in resistance to clindamycin and that would be concordant with the rise in the number of prescriptions of clindamycin for dogs, see “Use of antimicrobials”.

Noteworthy in this year's material is that resistance to cephalothin and oxacillin is higher compared to previous years, which mostly is due to cases of pyoderma caused by methicillin resistant *S. pseudintermedius* (MRSP). At SVA, all isolates of *S. pseudintermedius* with MIC of >0.5 mg/L for oxacillin are examined for *mecA* gene with PCR (see Appendix 3 for details). In this material 18 isolates were analysed with PCR and 12 (3%) were confirmed methicillin resistant. The new break-point for *S. pseudintermedius* (>0.25 mg/L) from CLSI in 2010 (<http://jvdi.org/cgi/data/21/1/53/DC1/1>) cannot be applied on this data because of the oxacillin range of the microdilution panels used. For further information on MRSP, see Highlight “Methicillin resistant *S. pseudintermedius* – an update”.

Multiresistance occurred in 34% of the isolates. Thirty six isolates were resistant to five or more antimicrobials (8%) and a third of them was MRSP. Resistance to penicillin, clindamycin and erythromycin was the most common phenotype, occurring in 72% of multiresistant isolates. Almost half of these were also resistant to tetracycline. Macrolide resistance in *S. pseudintermedius* is commonly mediated by *erm*-genes, and if these genes are constitutively expressed, the bacteria will be resistant also to lincosamides (clindamycin) and streptogramin B. In this material, 88% of isolates resistant to erythromycin were also resistant to clindamycin. Resistance to enrofloxacin occurred mainly in multiresistant phenotypes.

In this data, there is a high probability of bias towards dogs with recurrent skin infections, previously treated with anti-

TABLE DOG I. Resistance (%) in *Escherichia coli* from dogs 1992-2010 and distribution of MICs for isolates from 2010. Isolates are from clinical submissions of urine samples.

Antimicrobial	Resistance (%)							Distribution (%) of MICs (mg/L)									
	1992-94 n=245	1995-97 n=296	1998-00 n=418	2001-03 n=621	2004-06 n=917	2007-09 n=1527	2010 n=803	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	18	18	18	18	19	15	14				2.2	52.4	30.3	0.5	14.5		
Cefotaxime	-	-	-	-	-	<1	2			97.9	1.2	0.9					
Enrofloxacin ^a	9	9	10	9	10	8	8	92.4	3.1	2.0	0.7	0.1	1.6				
Gentamicin ^b	2	1	2	2	1	1	1				96.4	2.4	0.5	0.1	0.6		
Nitrofurantoin	3	3	1	2	2	1	1						97.6	1.1	1.2		
Polymyxin B	-	-	-	-	-	4	3				96.8	1.5	1.7				
Tetracycline	16	14	12	11	10	8	8				16.4	70.6	4.1	0.6	8.2		
Trim/Sulph ^c	9	8	11	13	15	9	5			93.8	0.9	0.2	0.1	5.0			

^a Cut-off value >0.25 mg/L until 2002; ^b Cut-off value >8 mg/L until 2001; ^c Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphametoxazole) and cut-off value >4 mg/L until 2001.

microbials that could explain the high levels of resistance. A prospective study by Holm et al., (2002) showed higher levels of multiresistance among isolates from recurrent compared to those from first-time pyoderma. Pyoderma is a common cause for dog owners to seek veterinary consultation and this condition is often treated with clindamycin or cephalosporins. Fortunately, the total number of prescriptions for dogs continues to decline (See Highlight "Use of antimicrobials, Dogs"). To be able to control the resistance situation in *S. pseudintermedius*, a prudent use of antimicrobials together with an effective infection control programme is of highest priority.

Pseudomonas aeruginosa

Isolates of *Pseudomonas aeruginosa* are from samples from the external ear canal. This species is considered inherently resistant to e.g. trimethoprim-sulphonamides, tetracyclines and aminopenicillins (including combinations with clavulanic acid).

Of the antimicrobials tested, fluoroquinolones, gentamicin and polymyxin B, are the only substances that would be effective for treatment of pseudomonal infections in dogs and therefore, the susceptibility data of these substances are presented in Table Dog III. All isolates were susceptible to polymyxin B. However, resistance to gentamicin or fluoroquinolones occurred and four isolates were resistant to both substances (1%).

In addition, the maximum plasma concentration (C_{max}) of the fluoroquinolones currently licensed for use in dogs in Sweden, after oral treatment at the label dosage, ranges from 1.5-2.5 mg/L. To have beneficial effect of treatment, the C_{max} to MIC ratio should preferably be >4 (Walker & Dowling, 2006). It is clear that the ratio would not be reached in most infection sites after systemic administration even for the more susceptible isolates. Local antimicrobial treatment in dogs with ear infections is of course recommended.

TABLE DOG II. Resistance (%) in *Staphylococcus pseudintermedius* from dogs 1992-2010 and distribution of MICs for isolates from 2010. Isolates are from clinical submissions of samples from skin.

Antimicrobial	Resistance (%)							Distribution (%) of MICs (mg/L)									
	1992-94 n=304	1995-97 n=322	1998-00 n=433	2001-03 n=382	2004-06 n=374	2007-09 n=859	2010 n=444	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Cephalothin	<1	<1	0	1	1	3	4					96.2	1.1	2.7			
Clindamycin	12	20	21	18	19	23	26				72.5		1.8	25.7			
Enrofloxacin	-	-	-	2 ^e	2	5	6	65.8	25.4	2.7	2.3	0.7	0.2	2.9			
Erythromycin ^a	21	28	27	24	26	28	30			69.4	0.7	0.2	0.4	29.3			
Fusidic acid	9	14	20	20	25	24	20					75.4	4.5	20.0			
Gentamicin	<1	<1	<1	0	1	3	3					96.4	0.7	0.7	1.1	1.1	
Nitrofurantoin	1	1	<1	1	<1	<1	2								97.3	1.1	1.6
Oxacillin	1	2	1	2	2	1	4			95.9	0.7	3.4					
Penicillin ^b	79	80	80	80	84	87	86										
Tetracycline	24	12	28	25 ^a	32	30	31				68.9	0.2		0.2	30.6		
Trim/Sulph ^c	1	2	1	3	6	5	6			77.2	16.2	0.9	0.4	5.2			

^a Cut-off value >4 mg/L until 2001; ^b Denotes beta-lactamase production; ^c Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole).

TABLE DOG III. Resistance (%) in *Pseudomonas aeruginosa* from dogs 2002-2003, 2009 and 2010, and distribution of MICs for isolates from 2010. Isolates are from clinical submissions of samples from the ear canal of dogs.

Antimicrobial	2002-03 n=234	2009 n=261	2010 n=313	Distribution (%) of MICs (mg/L)													
				≤0.12	0.25	0.5	1	2	4	8	16	32	>32				
Enrofloxacin	NA ^a	25	20	0.9	2.6	15.3	43.1	18.2	6.4	13.4							
Gentamicin	9	5	2					80.8	11.5	5.8	1.3	0.6					
Polymyxin B	-	0	0					96.8	3.2								

^a NA= not applicable because of the range of enrofloxacin concentrations tested.

Cat

Escherichia coli

Isolates of *Escherichia coli* are from samples of urine, submitted either as urine or as dip-slide cultures. Resistance to ampicillin was the most common trait (Table Cat I).

In 2010, 3% of the isolates were multiresistant (i.e. resistant to three or more substances). None of the isolates were resistant to five or more antimicrobials.

One isolate that had a MIC >1 mg/L for cefotaxime was confirmed being both ESBL and AmpC producing. Further

analyses are needed to clarify whether the AmpC gene is chromosomal or placed on a plasmid. For more information on resistance to 3rd generation cephalosporins see Highlight “*Enterobacteriaceae* producing extended spectrum beta-lactamases (ESBL) – isolates from diagnostic submissions”.

Cats with symptoms from the urinary tract are often treated with aminopenicillins or fluoroquinolones. This year, seven isolates were resistant to both these antimicrobials, i.e. about 3% of all isolates. However, bacterial urinary tract infections are rare in cats and other causative agents or underlying causes have to be investigated prior to antimicrobial treatment.

TABLE CAT I. Resistance (%) in *Escherichia coli* from cats 1992-2010 and distribution of MICs for isolates from 2010. Isolates are from clinical submissions of urine samples.

Antimicrobial	Resistance (%)						Distribution (%) of MICs (mg/L)									
	1992-97 n=61	1998-00 n=74	2001-03 n=135	2004-06 n=224	2007-09 n=546	2010 n=236	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	26	34	27	22	18	17				4.2	62.3	16.1	0.4	16.9		
Cefotaxime	-	-	-	-	3	1			98.7	0.8	0.4					
Enrofloxacin ^a	5	8	13	7	7	8	91.5	4.2	3.4	0.4			0.4			
Gentamicin ^b	0	3			1	<1					96.6	2.5	0.4		0.4	
Nitrofurantoin	2	2	1	3	1	1								98.3	0.4	1.3
Polymyxin B	-	-	-	-	6	3					97.5	0.4	2.1			
Tetracycline	28	16	16	14	8	6				22.9	64.0	7.2	0.4	5.5		
Trim-Sulph. ^c	7	10	15	7	5	4			95.8	0.4	0.4		3.4			

^a Cut-off value >0.25 (mg/L) until 2002; ^b Cut-off value >8 mg/L until 2001; ^c Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole), cut-off value >4 mg/L until 2001.



Methicillin resistant *Staphylococcus pseudintermedius* (MRSP) – an update

SINCE 1ST OF JANUARY 2008, methicillin resistant coagulase positive staphylococci are notifiable in Sweden. On suspicion on methicillin resistant *Staphylococcus pseudintermedius* (MRSP), diagnostic laboratories are advised to send the isolates to the National Veterinary Institute (SVA) for confirmation by PCR for the presence of *mecA* gene.

The first methicillin resistant *S. pseudintermedius* isolated in Sweden was from a healthy dog in a screening for methicillin resistant *S. aureus* in 2006. Later the same year 13 clinical isolates from postoperative wounds were confirmed, mainly from dogs sampled at two referral animal hospitals. In 2007, the number of confirmed cases was 77. Most of them had a characteristic antibiogram, being susceptible only to two substances of those licensed for therapy in veterinary medicine in Sweden; fusidic acid and tetracycline (SVARM 2007). In 2008, 78 MRSP from dogs and 4 from cats were confirmed. During that year, the first isolates with resistance to tetracycline were found. During 2009, 121 MRSP from dogs, seven MRSP from cats and one MRSP from a horse were confirmed. In 2010, 100 dogs and five cats were confirmed with MRSP.

In a survey financed by the Board of Agriculture, MRSP from 2007 and up to and including the first six months of 2010, will be examined for antimicrobial susceptibility, staphylococcal chromosomal cassette, spa-type, pulse field gel electrophoresis (PFGE) and multilocus sequence type (MLST). Results will be evaluated in relation to sampling site and geographical distribution.

Preliminary data from analyses of 219 (in total 245) isolates show that 94% of the MRSP were from dogs. Moreover, 40% of the MRSP were isolated from wounds followed by 27% from skin including samples from ear. A majority of the isolates (>90%) was resistant to macrolides, lincosamides, gentamicin, kanamycin, ciprofloxacin and trimethoprim. Resistance to chloramphenicol occurred in 45% of the isolates and 6% was resistant to tetracycline and 1% to fusidic acid. Thus a greater part has the characteristic antibiogram described above. However, one isolate was resistant to all the antimicrobials tested.

The relatedness between the isolates in Sweden is high, >90% belong to spa-type t02 and 86% have the same PFGE-profile. Further analyses of correlation between PFGE-profiles, spa-type, MLST and geographical distribution will be done. In addition, spa-type t02 is also the dominating spa-type in Europe as shown by Perreten and co-workers (2010), when over 100 MRSP from eight different countries, both in Europe and North America, were analysed. Representative MRSP from Sweden were included.

Since the first cases of MRSP, there have been active discussions among veterinarians on how to prevent further spread and how to correctly use antimicrobials. For instance, at many animal clinics and hospitals, infection control programs have been implemented with focus on strict hand hygiene routines. Also, veterinarians with special interest in dermatology have agreed on an antimicrobial policy for treatment of dogs with dermatological disorders.

Appendix 1: Demographic data

AGRICULTURAL STATISTICS are provided by Statistics Sweden in collaboration with the Board of Agriculture and published annually as a Yearbook of Agricultural Statistics and continuously as Statistical Messages (SM). The Yearbook and Statistical Messages are available on the Internet via the websites for Statistics Sweden (www.scb.se) or the Board of Agriculture (www.sjv.se).

Annual figures on number of animals and holdings are given in Table AP1 I & II, and on numbers and volumes of

animals slaughtered in Table AP1 III & IV. Details on methodology as well as comments on the data can be found in the respective data sources.

Briefly, the total number of dairy cows, pigs and laying hens has decreased notably over the last three decades concomitantly with an increase in herd size. In the same period, the number of beef cows, sheep and chickens reared for slaughter has increased. Data on horses are not available all years but since 2004 the number of horses has increased substantially.

TABLE AP1 I. Number of livestock and horses (in thousands) 1980-2010 (Yearbook of Agricultural Statistics Sweden 2001 & 2009 and Statistical Message JO 20 SM 1101 & JO 24 SM 1101).

Animal Species	1980 ^a	1985 ^a	1990	1995	2000	2005	2008	2009	2010
Cattle									
Dairy cows	656	646	576	482	428	393	357	357	348
Beef cows	71	59	75	157	167	177	196	192	197
Other cattle >1 year	614	570	544	596	589	527	513	502	512
Calves <1 year	595	563	524	542	500	508	492	488	478
Total, cattle	1 935	1 837	1 718	1 777	1 684	1 605	1 558	1 538	1 537
Pigs									
Boars & sows	290	260	230	245	206	188	170	160	156
Fattening pigs >20 kg ^b	1 254	1 127	1 025	1 300	1 146	1 085	974	943	937
Piglets <20kg ^c	1 170	1 113	1 009	769	566	539	465	426	427
Total, swine	2 714	2 500	2 264	2 313	1 918	1 811	1 609	1 529	1 520
Sheep									
Ewes and rams	161	173	162	195	198	222	251	254	273
Lambs	231	252	244	266	234	249	273	287	292
Total, sheep	392	425	406	462	432	471	525	540	565
Laying hens									
Hens	5 937	6 548	6 392	6 100	5 670	5 065	5 546	5 261	6 061
Chickens reared for laying	2 636	2 159	2 176	1 812	1 654	1 697	1 649	1 898	1 647
Total, hens	8 573	8 708	8 568	7 912	7 324	6 762	7 195	7 159	7 707
Turkeys									
Total, turkeys	-	-	-	-	-	122	-	-	130
Horses									
Total, horses	-	-	-	-	-	283 ^d	-	-	363

^a For 1980 and 1985 only cattle and sheep at premises with more than 2 ha counted; ^b Before 1995, the figure denotes pigs above 3 months of age; ^c Before 1995, the figure denotes pigs below 3 months of age; ^d Data from 2004.

TABLE AP1 II. Number of holdings with animals of different types, 1980-2009 (Yearbook of Agricultural Statistics, Sweden 2001 & 2009 and Statistical Message JO 20 SM 1101 & JO 24 SM 1101).

Animal Species	1980	1985	1990	1995	2000	2005	2008	2009	2010
Cattle									
Dairy cows	44 143	35 063	25 921	17 743	12 676	8 548	6 474	6 020	5 619
Beef cows	12 436	10 310	10 883	17 069	13 861	12 821	12 345	11 922	12 190
Other cattle >1 year	63 179	52 652	42 696	39 160	30 457	24 808	21 536	20 330	20 295
Calves <1 year	62 314	52 001	41 986	36 542	27 733	22 888	19 911	18 965	18 494
Total holdings with cattle	70 503	58 872	47 292	41 990	32 063	26 179	22 844	21 733	21 586
Sheep	10 238	10 595	9 749	10 037	8 089	7 653	8 186	8 245	8 657
Pigs	26 122	19 937	14 301	10 753	4 809	2 794	2 380	2 027	1 695
Laying hens	23 603	17 531	12 900	9 593	5 678	4 916	4 643	3 306	3 703
Chickens reared for laying	5 093	2 714	1 875	1 405	715	634	854	573	487
Broilers	-	-	-	-	-	234	198	183	181
Turkeys	-	-	-	-	-	383	-	-	102
Horses	-	-	-	-	-	56 000 ^a	-	-	78 000

^a Data from 2004.**TABLE AP1 III.** Number of animals slaughtered (in thousands) at slaughterhouses, 1980-2009. (Yearbook of Agricultural Statistics, Sweden 1981, 1986, 1991 & 2009 and Statistical Message JO 48 SM 1102).

Animal Species	1980	1985	1990	1995	2000	2005	2008	2009	2010
Cattle									
Cattle >1 year	574	584	523	502	490	433	401	430	426
Calves <1 year	130	152	70	30	39	33	29	29	27
Total, cattle	704	736	593	532	529	466	430	459	452
Pigs	4 153	4 283	3 653	3 743	3 251	3 160	3 072	2 956	2 946
Sheep	302	328	280	189	202	206	235	255	255
Broilers	40 466 ^a	36 410 ^a	38 577 ^a	61 313	68 617	73 458	75 087	73 504	78 507
Turkeys	-	-	-	-	-	-	471	477	495

^a Data supplied by the National Food Administration.**TABLE AP1 IV.** Quantity of livestock slaughtered (in 1000 tonnes) at slaughterhouses, 1990-2009 (Yearbook of Agricultural Statistics, Sweden 1991 & 2009 and Statistical Message JO 48 SM 1102).

Animal Species	1990	1995	2000	2004	2005	2008	2009	2010
Cattle								
Cattle >1 year	139.5	140.1	145.4	137.8	131.4	124.5	135.4	133.7
Calves <1 year	6.8	3.2	4.4	4.6	4.5	4.3	4.6	4.3
Total, cattle	146.3	143.3	149.8	142.4	135.9	128.8	140.0	138.0
Pigs	293.1	308.8	277.0	294.5	275.1	270.7	261.7	264.1
Sheep	5.0	3.5	3.9	3.8	4.1	4.6	5.1	5.0
Broilers	44.0 ^a	73.6 ^a	89.9	91.2	96.2	107.2	105.2	112.0
Turkeys	-	-	-	-	-	2.7	2.8	2.9

^a Data supplied by the National Food Administration.

Appendix 2: Materials and methods, use of antimicrobials

Legal framework and distribution of medicines

Marketing of drugs in Sweden is regulated by the Medicinal products act, which applies both to human and veterinary medicinal products (VMPs). According to this Act, a medicinal product may not be sold until it has been granted marketing authorisation by the Medical Products Agency (MPA). In case there are no authorised veterinary medicinal products for a certain condition, the MPA can permit special license prescription for a VMP for a specified pharmacy and prescriber. VMPs have to be dispensed through pharmacies, which are supplied by drug wholesalers or manufacturers. Veterinarians are not allowed to sell VMPs but may deliver products to the animal care-taker in relation to examination of a case, however, for self cost (no profit). Veterinarians are not permitted to own a pharmacy.

Antimicrobial drugs for veterinary use, including medicated feed, may only be sold on prescription.

All pharmacies in Sweden are required to provide prescription statistics on a daily basis to an infrastructure company owned by the state: Apotekens Service AB. For VMPs, the animal species as given on the prescription is also recorded, unless the product is sold for use in veterinary practice. Apotekens Service maintains a database and provides statistics to the competent national and regional authorities and to others on a commercial basis.

Feed mills may only mix antimicrobials in feed if they are controlled and authorised by the Swedish Board of Agriculture (SBA). The feed mills normally acquired the antimicrobial VMPs from a pharmacy. All quantities of VMPs used by feed mills are reported yearly to the SBA as part of the feed control. Mixing of antimicrobials in feed may also take place on farms; provided that the SBA has inspected and authorised the establishment for the purpose. In such cases, the premix is sold by a pharmacy following prescriptions from a veterinarian.

Data sources and inclusion criteria

Raw data on sales is obtained from Apotekens Service and represent the sales of antimicrobial VMPs sold by pharmacies.

For the overall statistics (Table AC I-III), the data include all antimicrobial VMPs marketed in Sweden and sold for use in terrestrial animals in the ATCvet classes QA, QG and QJ. Most antimicrobial VMPs sold with special license (products prescribed and sold on exemption from general Swedish market authorization) are also included. Medicinal products authorised for human use but prescribed for use in animals is not included in this section.

The ionophoric antibiotics are presently regulated as feed additives and not sold through pharmacies and are not

included in the statistics from Apotekens Service. However, the SBA collects figures on sales of ionophores from the feed mills as a part of the feed control system. As the source differs, data on ionophores are given only in Table AC III.

Data for year 2009 published by the SBA is used to present the repartition of the sales per category of animals (Table IV) (www.jordbruksverket.se). The data include VMPs in the same ATCvet classes as given above and in addition products authorised for human use but sold for use in animals for the corresponding classes are included. The attribution to species is done using data from electronic records prescriptions from pharmacies. Efforts are made to assign the sales of products sold for use in veterinary practice to species or to a category of animals (companion or food producing animals) as far as possible, using information on e.g. which animal species a particular product is authorised for.

The electronic records on animal species are also used to obtain data on number of prescriptions and packages sold specifically for use in dogs. That data-set closely corresponds what is called "out-patient use".

The data coverage is assumed to be close to 100%. In rare cases, premixes mixed in medicated feed may be delivered from feed mills without the sales being recorded by a pharmacy. Examination of the reports by all feed mills to the SBA shows that this happened only once during 2005-2009 (a total quantity of 40 kg active substance). In addition, as mentioned small, quantities of drugs sold on special licence might not have been captured.

Analysis and reporting of data

Data are retrieved as number of packages sold per product presentation and per animal species, if recorded. Calculation to kg active substance is done based on product information obtained from the national product register of the MPA.

Data on sales of antimicrobials for animals has been analysed and reported by the SVA since 1980. SVA is responsible for monitoring antimicrobial resistance but not of use, but still monitors use to support work on its mandate to stimulate rational use of antimicrobials. Statistics on usage is published in English in the yearly reports of the Swedish Veterinary Antimicrobial Resistance Monitoring programme. Since 2005, the Board of Agriculture is responsible for statistics on use of certain veterinary medicinal products, including antimicrobials. SBA publishes the results in Swedish in an electronic report on sales of certain drugs for animals. Data are reported by companion or production animals (including horses), and to the extent possible also by specific animal species.

Appendix 3: Materials and methods, resistance monitoring

Sampling strategy

Zoonotic bacteria

Salmonella

Salmonellosis in animals is a notifiable disease in Sweden and one isolate from each notified incident must be confirmed at SVA. Data presented in SVARM 2010 include one isolate of each serovar, and when appropriate phage-type, from each warm-blooded animal species in each incident notified 2010. In cats, a randomly selected subset of isolates was tested. In addition, isolates from incidents previously notified and still under restrictions 2010 are included. Also included are isolates obtained in the salmonella surveillance programme from samples collected at slaughter (carcass swabs, neck skins and lymph nodes).

Campylobacter

Campylobacter spp. were isolated from caecum content from healthy broiler chickens sampled at slaughter within the Swedish Campylobacter programme in which whole caeca are collected from each batch of broilers slaughtered. In 2010, 441 flocks were positive for Campylobacter. From these, 100 isolates of *Campylobacter jejuni*, each representing one flock were randomly selected for susceptibility testing. The isolates were stored in -70°C until testing.

Methicillin resistant *Staphylococcus aureus* (MRSA)

Isolates of suspected MRSA were sent from veterinary clinical laboratories in Sweden to the Dept. of Animal Health and Antimicrobial Strategies, Section for Antibiotics, SVA, for confirmation. In the screening for MRSA in horses, 284 randomly selected horses were sampled with nasal swabs on admission to five different animal hospitals and clinics from November 2010 to early February 2011. In the screening for MRSA in pigs, nasal swabs were taken at six slaughter houses in Sweden during May and June 2010. Five pigs per herd were sampled directly after slaughter. Altogether, 191 herds were sampled and the samples were transported cooled to the laboratory where samples from the same herd were pooled.

Indicator bacteria

Broilers

Indicator bacteria, *E. coli* and *Enterococcus* spp., were isolated from caecal content of healthy broilers sampled at slaughter. Samples cultured were from the Swedish Campylobacter programme—see above. From these samples, 100 were selected by convenience in February-May and 100 in September-November for culture. Each sample is from a unique flock but not always from a unique production site. Samples cultured were collected at seven abattoirs that in 2010 accounted for >99% of the total volume of broilers slaughtered. The number

of samples from each abattoir is roughly proportional to the annual slaughter volume of the abattoir.

Broiler meat

Broiler meats were sampled in cooperation with SVA, SLV (National Food Administration) and the Swedish Poultry Meat Association. Sample from 100 frozen broiler filets were collected from 100 packages and from different batches. The number of samples from each abattoir were proportional to the slaughter volume.

Horses

In total, 284 randomly selected horses were sampled with rectal swabs on admission to five different animal hospitals from November 2010 to early February 2011. In addition, on 17 different stud farms 147 mares were randomly selected and sampled with rectal swabs from May to July 2010.

Animal pathogens

Isolates of animal pathogens included are from routine bacteriological examinations of clinical submissions or post-mortem examinations. Isolates of *Actinobacillus pleuropneumoniae* from pigs and part of the isolates of *Pasteurella* spp. from calves were, however, isolated from samples collected in surveys initiated within SVARMpat.

In pigs, *E. coli* are isolated from the gastro-intestinal tract (gut content, faecal samples or mesenteric lymph nodes), *Brachyspira* spp. from faecal samples and *Pasteurella* spp. from nasal swabs collected within a control programme for atrophic rhinitis in nucleus and multiplying herds. *Actinobacillus pleuropneumoniae* are isolated from tissue samples from lungs sampled post mortem. *Pasteurella* spp. from cattle are isolated from the respiratory tract. In horses, *E. coli* are isolated from the genital tract of mares, *Streptococcus zooepidemicus* from the respiratory tract and *S. aureus* from skin samples. In dogs, *E. coli* are isolated from samples of urine, *S. pseudintermedius* from skin samples and *Pseudomonas aeruginosa* from samples from the external ear. *E. coli* from cats are isolated from samples of urine. In farmed fish, *Aeromonas salmonicida* subsp. *achromogenes*, *Flavobacter columnare* and *Flavobacter psychrophilum* are from post mortem examination.

Isolation and identification of bacteria

Zoonotic bacteria

Salmonella

Salmonella were isolated and identified at the Dept. of Bacteriology, SVA or at regional laboratories in accordance with standard procedures. All samples within official control programmes are cultured according to the procedures detailed by the MSRV (ISO-EN 6579:2002/ Amd 1:2007). Confirmatory identification and serotyping of isolates was

performed at the Dept. of Bacteriology, SVA according to the standard procedures of Kaufmann and White. The Dept. of Bacteriology, SVA is accredited for isolation, identification and serotyping of *Salmonella*.

Isolates of *Salmonella* Typhimurium and *S. Enteritidis* were phage-typed by the Swedish Institute for Infectious Disease Control (SMI), Stockholm using the Colindale scheme.

Campylobacter

Campylobacter spp. from broilers were isolated and identified at the Dept. of Bacteriology, SVA. Samples were cultured for thermophilic *Campylobacter* spp. according to ISO 10272-1:2006 with direct cultivation on mCCDA and incubation at 42°C. Identification was based on colony morphology, microscopic appearance including motility and the following phenotypic characteristics: production of oxidase, catalase, hippurate hydrolysis reaction and indoxyl-acetate reaction (Nachamkin, 1999). With these tests, hippurate-positive *C. jejuni* were identified. The Dept. of Bacteriology, SVA is accredited for isolation and identification of *Campylobacter* spp.

Methicillin resistant *Staphylococcus aureus* (MRSA)

Screening samples from horses were incubated over night at 37°C in 10 mL trypton soy broth with 4% NaCl, 1% mannitol, 3 mg/L cefoxitin and 50 mg/L aztreonam. Pools of five screening samples from the same pig herd were incubated over night at 37°C in 50 ml Mueller-Hinton broth with 6.5% NaCl. From this pre-enrichment broth, one mL was inoculated into 9 mL trypton soy broth with 3.5 mg/L cefoxitin and 75 mg/L aztreonam and incubated over night at 37°C. From both samples from horses and pigs, 10 µL of the selective broth were spread on bovine-blood agar and on Oxoid MRSA Brilliance-agar. The plates were incubated over night at 37°C. Suspected colonies from the MRSA agar were spread on bovine-agar plates and incubated over night at 37°C. Suspected colonies were confirmed with genotypic methods.

Indicator bacteria

Escherichia coli

Approximately 0.5 g of caecum content from broilers was diluted in 4.5 mL saline. After thorough mixing, 0.1 mL of this suspension was spread on MacConkey agar and MacConkey agar with cefotaxime 1mg/L and incubated overnight at 37°C.

Twenty-five g of broiler meat was homogenized thoroughly in 2 min with 225 mL BPW (Buffered Pepton Water). Five mL was thereafter transferred to 45 mL MacConkey broth and incubated at 37°C for 18-24 h. From the pre-enrichment 10µL was spread on MacConkey agar and incubated overnight at 37°C.

One lactose positive colony with morphology typical for *E. coli* was sub-cultured onto horse-blood agar (5% v/v), after which the isolate was tested for production of tryptophanase (indole) and b-glucuronidase (p-nitrophenyl-b-D- glucopyranosiduronic acid, PGUA). Only lactose-positive isolates with typical morphology and positive reactions in both tests were selected for susceptibility tests. Colonies growing on

MacConkey agar with cefotaxime were sub-cultured on horse-blood agar (5% v/v) and further tested for ESBL detection.

Enterococci

Caecum content from broilers was diluted as described for *E. coli* and cultured on solid media without antibiotics and on selective plates with vancomycin (16 mg/mL).

Ten mL of the BPW from homogenised broiler meat (above) was mixed with 10 mL Enterococcosel broth and 10 ml of BPW to 10 mL double concentrated Enterococcosel broth with vancomycin 32mg/L, incubated at 37°C over night.

Culture without selective antibiotics: Diluted caecum content (0.1 mL) was spread onto Slanetz-Bartley (SlaBa) agar. The plates were incubated for 48 h at 37°C. From the Enterococcosel broth 10 µL was cultured on SlaBa agar and incubated at 37°C for 48 h. Two colonies, randomly chosen, were sub-cultured on bile-esculin agar and blood agar (37°C, 24 h). Colonies with morphology consistent with enterococci, and with a positive reaction on bile-esculin agar were identified to species level according to Devriese et al. (1993) by use of the following biochemical tests: mannitol, sorbitol, arabinose, saccharose, ribose, raffinose and methyl-a-D-glucopyranoside. *E. faecium* and *E. faecalis* were tested for antimicrobial susceptibility.

Selective culture for vancomycin resistant enterococci: Diluted caecum content (0.1 mL) was cultured on SlaBa agar with vancomycin (16 mg/L) and incubated at 37°C for 18-24 h. From the Enterococcosel broth with vancomycin, 10 µL was cultured on SlaBa agar with vancomycin (16 mg/L) and incubated at 37°C for 18-24 h.

From plates showing growth of colonies typical for enterococci, one colony was sub-cultivated on bile-esculin agar and blood agar (37°C, for 24 h). Identification and susceptibility testing of presumptive enterococci was performed as above.

Animal pathogens

Animal pathogens were isolated and identified with accredited methodology, following standard procedures at SVA. Bacteria from terrestrial animals were isolated at the Dept. of Bacteriology, and bacteria from fish at the Dept. of Animal Health and Antibiotic Strategies.

Susceptibility testing

Microdilution

The Dept. of Animal Health and Antimicrobial Strategies or the Dept. of Bacteriology performed antimicrobial susceptibility tests on bacteria from terrestrial animal, with accredited methodology, using dilution methods in cation adjusted Mueller-Hinton broth (CAMBH). Tests were performed following the standards for microdilution of the Clinical and Laboratory Standards Institute (CLSI, 2008). The microdilution panels used, VetMIC™, are produced at the Dept. of Vaccines and Blood products, SVA. Different panels were used depending on the bacterial species tested and the original purpose of the investigation (monitoring or clinical diagnostics). Minimum inhibitory concentration (MIC) was recorded

as the lowest concentration of the antimicrobial that inhibits bacterial growth.

The Dept. of Animal Health and Antibiotic Strategies performed antimicrobial susceptibility tests on bacteria from fish, using the same methodology as described above but adapted for aquatic bacteria according to Alderman & Smith (2001), which e.g. implies incubation at 20°C for two days.

For susceptibility testing of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*, a broth dilution method was used (Karlsson et al., 2003). The antimicrobials were dried in serial twofold dilutions in the tissue culture trays with 48 wells per plate. The wells were filled with 0.5 mL of a suspension of bacteria in brain heart infusion broth with 10% foetal calf serum (1×10^6 - 5×10^6 CFU/ml). The trays were incubated in an anaerobic atmosphere at 37°C for four days on a shaker.

Screening for methicillin resistance in *S. aureus* from milk samples from cows was performed with microdilution according to CLSI (2008), testing oxacillin with 2% NaCl added to the broth, and oxacillin without added NaCl and cefoxitin.

Phenotypic confirmatory test for production of extended spectrum beta-lactamases (ESBLs) in *E. coli* was performed by the double disc diffusion test according to CLSI (2008).

Genotyping

Suspected isolates of *S. aureus* was confirmed by polymerase chain reaction (PCR) identifying the *nuc* gene (Sasaki et al. 2010). Presence of the *mecA* gene in *S. aureus* and *S. pseud-intermedius* was confirmed by PCR according to Nilsson et al. (2005) in isolates with a phenotype indicating methicillin resistance.

Spa typing, a single locus sequence typing method using the polymorphic region X of the protein A gene, was performed on all isolates confirmed as MRSA. It was performed according to the method described by Harmsen et al. (2003) and the specific *spa* type was determined using BioNumerics® (Applied Maths).

Genotypic screening of ESBL and AmpC positive *E. coli* was performed by using Identibact Array Tube test according to the manufacturer (www.identibact.com). The test allows detection of the most common resistance genes of gram-negative isolates (Anjum et al., 2007). PCR was complementary performed for identification of plasmid-mediated AmpC and CTX-M mediated ESBL according to Perez-Perez and Hanson (2002) and Woodford et al. (2006).

In sixteen enterococcal isolates, selected by convenience, with MICs of vancomycin above >128 mg/L, the resistance genotype was confirmed with PCR for the *vanA* gene according to Dutka-Malen et al. (1995).

Quality assurance system

The Dept. of Animal Health and Antimicrobial Strategies and Dept. of Bacteriology are accredited according to SS-EN ISO/IEC 17025 by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC) to perform antimicrobial susceptibility tests with microdilution methods. The Dept. of Bacteriology is also accredited for isolation and identification of animal pathogens and *Salmonella* according to the same standard.

For susceptibility tests of zoonotic, pathogen and indicator bacteria, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* CCUG15915 (analogue to ATCC 29213) and *Campylobacter jejuni* CCUG 11284 (analogue to *Campylobacter jejuni* ATCC 33560) were included as quality controls. Relevant control strains were also included and evaluated at least once weekly for animal pathogens. For testing of *Brachyspira*, the *B. hyodysenteriae* type strain B78^T ATCC 27164^T was used for quality control.

The Dept. of Animal Health and Antimicrobial Strategies participates in several proficiency tests for antimicrobial susceptibility testing. These are arranged either by the European Union Reference Laboratory - Antimicrobial resistance or as national studies. Likewise, the Dept. of Bacteriology participates in proficiency tests concerning isolation and identification of *Salmonella* spp. and general clinical veterinary bacteriology and susceptibility tests.

Data handling

Records on *Salmonella* and animal pathogens such as source of cultured sample, identification results, antimicrobial susceptibility etc. are registered in a database at SVA. Relevant data were extracted for evaluation and compilation. For indicator bacteria data was recorded in an Access database at the Dept. of Animal Health and Antimicrobial Strategies.

Calculations and analysis of data were performed in the computer programs Microsoft Access or Microsoft Excel.

Concerning confidence limits

When the prevalence of antimicrobial resistance is close to zero, e.g. when one out of 120 isolates is resistant, the question arises how to calculate the prevalence of resistance and its confidence intervals. In the example, the prevalence could be estimated to 0.83% while the 95% confidence interval is trickier. The normal approximation to the binomial distribution would give a lower confidence of -0.8% and an upper confidence limit of 2.5%. The lower limit is nonsensical and indicates the unsuitability of the normal approximation in this case.

One way out of the dilemma is to calculate the exact binomial confidence limits, which would be possible in some cases (small number of isolates). Another alternative is to run Monte-Carlo simulations based on the beta-distribution which is possible but quite laborious for a huge set of data since each prevalence estimate has to be simulated 10 000 times. Finally the relationship between the F-distribution, the beta-distribution and the binomial distribution can be used. This gives the formulae that enable calculations of the confidence interval (Rao, 1965). Using this approach, the confidence intervals in the example would be 0.021% and 4.6%.

In conclusion, the normal approximation to the binomial distribution might be unsuitable when the prevalence is close to 0% or close to 100% since the approximation might lead to confidence intervals lower than 0% or higher than 100%. Moreover, when the prevalence of resistance is less than 5% using the link between the F-distribution and the binomial distribution yield different confidence intervals compared to those obtained from the normal approximation and should accordingly be preferred.

Appendix 4: Cut-off values for resistance

FOR INTERPRETATION of results of susceptibility testing of zoonotic bacteria (*Salmonella*) and indicator bacteria (*Escherichia coli* and enterococci) epidemiological cut-off values (ECOFF) issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (<http://www.escomid.org>) were used. When no ECOFFs are issued by EUCAST, values based on MIC distributions obtained in the SVARM programme were used. This approach was also used for ciprofloxacin in *E. coli* because the recommended cut-off value (>0.03 mg/L) cuts through distributions of MICs in SVARM in a manner not in agreement with the concept of wild-type distributions.

ECOFFs were used when available also for animal pathogens. When no ECOFFs were available, or the range of concentrations tested was inappropriate for the recommended value, values based on MIC distributions obtained in the SVARM programme were used. Clinical breakpoints issued by CLSI (2009) were also taken into consideration. Epidemiological

cut-off values classify isolates with acquired reduced susceptibility as resistant, which is relevant for monitoring purposes, but it should be understood that this not always implies clinical resistance.

Bacitracin values in this report are given in units/mL. In an attempt to convert unit/mL to mg/L we discovered that there appears to be some confusion in the matter. The bacitracin compound used in SVARM is obtained from Sigma and meets the standards set by the United States Pharmacopoeia (USP), stating that one unit is equivalent to 26 µg of the US standard. However, according to the International Standard Preparations, one international unit is equivalent to 13.51 µg. On the other hand, if the bacitracin is of a very high degree of purity, though unstable, it correspond to 66 (-70) units/mg, that is, one unit is equivalent to approximately 15 µg. Feedingstuff grade of bacitracin correspond to 42-50 units/mg (one unit=20-24 µg) (Otten et al., 1975).

TABLE AP4. Cut-off values (mg/L) for resistance. Values in red are current (April 2011) EUCAST epidemiological cut-off values (ECOFFs), blue underlined values deviate from ECOFFs and for values in black, ECOFFs are not defined.

Antimicrobial	<i>Actinobacillus pleuropneumonia</i>	<i>Brachyspira hyodysenteriae</i>	<i>Brachyspira pilosicoli</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>	<i>Enterococcus faecalis</i>	<i>Enterococcus faecium</i>	<i>Escherichia coli</i> (indicator)	<i>Escherichia coli</i> (pathogen)	<i>Pasteurella</i> spp.	<i>Pseudomonas aeruginosa</i>	<i>Salmonella enterica</i>	<i>Staphylococcus pseudintermedius</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus zooepidemicus</i>
Ampicillin	>1			>8	>8	>4	>4	>8	>8	>1		>8			>8
Bacitracin ^a						>32	>32								
Cefotaxime	>0.06							>0.25	>0.5	>0.06		>0.5			
Cefoxitin														>4	
Ceftiofur	>0.25							>1	>1	>0.25		>2		>2	
Cephalothin													>2	>1	
Chloramphenicol	>2					>32	>32	>16		>2		>16		>16	
Ciprofloxacin	>0.06			>1	>1			>0.06		>0.06		>0.06		>1	
Clindamycin													>4	>0.25	
Enrofloxacin				>0.5	>0.5			>0.12	>0.12	>0.25	>2	>0.25	>0.5	>0.5	
Erythromycin				>4	>16	>4	>4						>1	>1	
Florfenicol	>16							>16	>16	>16		>16		>8	>8
Fusidic acid													>4	>0.5	
Gentamicin	>8			>1	>2	>32	>32	>2	>4	>8	>8	>2	>4	>2	
Kanamycin						>1024	>1024	>8				>16		>8	
Linezolid						>4	>4								
Nalidixic acid	>16			>16	>32			>16		>16		>16			
Narasin						>2	>2								
Neomycin									>8			>4			
Nitrofurantoin									>32				>32		
Oxacillin													>1	>1	
Penicillin	>1									>1				^c	>1
Polymyxin B									>2		>4				
Spiramycin														>16	>16
Streptomycin	>32			>2	>4	>512	>512	>16	>16	>32		>16		>16	
Sulphametoxazole	>256							>256				>256			
Tetracycline	>2			>2	>2	>4	>4	>8	>8	>2		>8	>8	>1	>8
Tiamulin		>2	>2												
Trimethoprim	>4							>2	>2	>4		>2		>2	
Trim & sulpha ^b								>1	>1	>4		>0.5	>2	>0.5	>4
Tylosin		>16	>16												
Tylvalosin			>4												
Vancomycin						>4	>4								
Virginiamycin						>32	>4								

^a MIC in U/mL; ^b Concentration of trimethoprim given, tested with sulphametoxazole in concentration ratio 1/20; ^c beta-lactamase production.

Appendix 5: Antimicrobial agents licensed

ANTIMICROBIALS licensed for use in veterinary medicine in Sweden year 2010 are listed in Table AP5. Only substances in pharmaceutical preparations for systemic, oral, intrauterine or intramammary use are included (ATCvet codes QJ, QG, QA and QP). Data from FASS VET. 2010. For explanation of ATCvet code, see Appendix 2.

Changes since 2009

■ New substances or indications

Gamitromycin (QJ01FA95), injectable for use in cattle

■ Withdrawn substances or indications

Sulphaclozin (QP51A G04), oral solution for use in poultry.

Penicillin G, penetamathydroiodide (QJ01C E90), injectable for use in cattle.

Sulphadiazine/Trimethoprim (QJ01E W10), tablets for oral use in dogs.

Ibafloxacin (QJ01M A96), oral gel for use in dogs and cats. Difloxacin (QJ01M A94), tablets for oral use in dogs.

TABLE AP5. Antimicrobials licensed for use in cattle, sheep, pigs, poultry, horses, dogs and cats in Sweden, 2010. Routes of administration indicated: O = oral; I = injection; U = intrauterine; M = intramammary.

Antimicrobial agent	ATCvet code	Animal species						
		Cattle	Sheep	Pigs	Poultry	Horses	Dogs	Cats
Tetracyclines								
Doxycycline	QJ01A A02			O			O	O
Oxytetracycline	QJ01A A06, QG01A A07	I O U	I U	I O U	O			
Beta-lactams, penicillins								
Ampicillin	QJ01C A01	O		O		O	O	O
Amoxicillin	QJ01C A04	I		I			I O	O
Amoxicillin/Clavulanic acid	QJ01C R02			I			I O	I O
Penicillin G, sodium	QJ01C E01	I		I		I		
Penicillin G, procaine	QJ01C E09/QJ51C E09	I M	I	I		I	I	I
Beta-lactams, cephalosporins								
Cephalexin	QJ01D B01						O	
Ceftiofur	QJ01D D90	I						
Cefovecin	QJ01D D91						I	I
Sulphonamides/Trimethoprim								
Sulphadiazine/Trimethoprim	QJ01E W10	I	I	I		I O		
Sulphadoxine/Trimethoprim	QJ01E W13	I		I		I		
Macrolides								
Spiramycin	QJ01F A02	I						
Tulathromycin	QJ01FA94	I		I				
Gamitromycin	QJ01FA95	I						
Tylosin	QJ01F A90	I		I O	O		I	I
Lincosamides								
Clindamycin	QJ01F F01						O	O
Aminoglycosides								
Gentamicin	QJ01G B03					I U	I	I
Dihydrostreptomycin (DHS)	QA07A A90	O U	O U	O U		O U	O	O
Fluoroquinolones								
Danofloxacin	QJ01M A92	I						
Enrofloxacin	QJ01M A90	I		I	O		I O	I O
Marbofloxacin	QJ01M A93						O	O
Pleuromutilins								
Tiamulin	QJ01X X92			I O				
Valnemulin	QJ01X X94			O				
Combinations								
Penicillin G, procaine/DHS	QJ01R A01, QJ51R C23	I M	I	I		I	I	I
Penicillin G, benzatin/DHS	QJ51R C24	M						
Penicillin G, ester/Framycetin	QJ51R C25	M						
Penicillin G, ester/DHS	QJ51R C25	M						

Appendix 6: References

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Appendix 7: SVARM 2000-2010 - an overview

Data on antimicrobial susceptibility of over 20 000 isolates of bacteria have been presented in SVARM since 2000. The annual number of isolates of different categories is presented below.

TABLE AP7 I. *Salmonella*, number of isolates 2000-2010.

Source	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Warm-blooded animals	67	52	49	101	68	105	101	112	122	117	82
Cold-blooded animals										17	

TABLE AP7 II. *Campylobacter*, number of isolates 2000-2010.

Source	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Cattle		67					68				
Pig		98		105		100	46		97		
Broiler		50	100		100				38		100
Raw meat		74									
Water		19									

TABLE AP7 III. Indicator *Escherichia coli*, number of isolates 2000-2010.

Source	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Cattle	293						314			223	
Pig	260	308		303		390		342	349		
meat									19		
Broiler	274	296	306		300			296			181
meat											77
Horse											274
Dog							257				
Willow grouse						19					
Wild boar			87								
Sheep									115		

TABLE AP7 IV. Indicator *Enterococcus (faecium, faecalis and hirae)*, number of isolates 2000-2010.

Source	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Cattle	220						258			197	
Pig	210	235		282		226			218		
meat									20		
Broiler	226	280	291		245			284			171
meat											98
Horse											61
Dog							186				
Wild boar			56								
Sheep									77		

TABLE AP7 V. Animal pathogens, number of isolates 2000-2010.

Animal species & bacterial species	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Cattle											
<i>Escherichia coli</i> (enteric)			220		87	39	24			40	15
<i>Escherichia coli</i> (udder)				169							
<i>Klebsiella</i> spp. (udder)				44			24				
<i>Pasteurella</i> spp.	254			100				27	32	14	27
<i>Staphylococcus aureus</i> (udder)		100	100			96			87		
<i>Streptococcus dysgalactiae</i> (udder)			100								
<i>Streptococcus uberis</i> (udder)			100								
<i>Fusobacterium necrophorum</i>										41	
Pig											
<i>Actinobacillus pleuropneumoniae</i>	18							84	39	24	39
<i>Brachyspira hyodysenteriae</i>	50	75	109	100		31	26	23	15	24	9
<i>Brachyspira pilosicoli</i>				93		57	72	44	31	24	13
<i>Escherichia coli</i> (enteric)	399	82	340	340	386	325	298	93	83	102	94
<i>Pasteurella</i> spp.		75						38	25	24	10
<i>Staphylococcus hyicus</i>					20						
Poultry (laying hens)											
<i>Escherichia coli</i> (infection)								70			
Sheep											
<i>Staphylococcus aureus</i> (udder)								25			
<i>Fusobacterium necrophorum</i>										24	
Fish											
<i>Aeromonas salmonicida</i> subsp. achrom.								67	20	23	8
<i>Flavobacter columnare</i>								30	16	10	5
<i>Flavobacter psychrophilum</i>								42	27	24	21
Horse											
<i>Actinobacillus</i> spp.		40									
<i>Escherichia coli</i> (genital)	323	103	166	188	188	161	124	273	174	210	236
<i>Rhodococcus equi</i>	73	20			187						
<i>Streptococcus zooepidemicus</i>	301	174	163	150	185	175	174	180	159	152	43
<i>Staphylococcus aureus</i>										308	131
Dog											
<i>Escherichia coli</i> (urinary)	185	183	204	234	247	304	366	425	503	599	803
<i>Pasteurella multocida</i>					231						
<i>Pseudomonas aeruginosa</i>				234						261	313
<i>Staphylococcus pseudintermedius</i>	145	156	133	102	159	126	89	220	258	381	444
Cat											
<i>Escherichia coli</i> (urinary)			46	52	55	74	95	131	170	245	236



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