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Swedish Veterinary Antimicrobial Resistance Monitoring






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Preface

The problem with antibiotic resistance in Sweden is still limited. However during the last year several reports have been presented revealing that the situation, in some countries, is alarming. In November 2001 an EU-recommendation was adopted in which every member state is asked to put in place specific strategies on prudent use of antimicrobial agents. These strategies should comprise measures in relation to surveillance of antimicrobial resistance, surveillance of antimicrobial use, control and preventive measures, education and training, and research. Such a system has been in place in Sweden since 1995 through the Swedish Strategic Programme for the Rational Use of Antibiotics and Surveillance of Resistance (STRAMA), which is financially supported by the Swedish Government.

It is today generally accepted that all use of antimicrobials in different sectors contributes to the development of resistance. Therefore, in Sweden, human and veterinary medicine have collaborated over a number of years, not least within STRAMA. Based on this experience we are convinced that joint efforts between human and veterinary medicine are essential in order to counteract the threat that antimicrobial resistance poses to both human and animal health. Overall, the figures in this report indicate that the Swedish strategy in human and veterinary medicine has been successful in containing resistance.

The ultimate goal is to preserve the effectiveness of available antimicrobials for man and animals and the general concept is to use antimicrobials only when needed, on prescription only and that the choice of treatment is based on relevant information. In addition further efforts must be made to prevent infectious diseases both in human and in veterinary medicine.

In this document we have combined our annual reports: SWEDRES, Swedish Antibiotic Utilisation and Resistance in Human Medicine and SVARM, Swedish Veterinary Antimicrobial Resistance Monitoring. Our hope is that the report will serve as a basis for further policy recommendations and intervention strategies, and that it will increase our understanding of the dynamics of resistance.

Summary

This third report from SVARM concurs with the two previous reports and other Swedish studies in this field and indicates that the situation regarding antimicrobial resistance in bacteria of animal origin is stable. Resistance does occur but in an international perspective the levels are low. To sustain the favourable situation, it is important to uphold the tradition of prudent use of antimicrobials in animals and a good animal health status.

The information gathered in programmes like SVARM, monitoring both antimicrobial consumption and occurrence of resistance, should further the understanding of the epidemiology of antimicrobial resistance. From the data gathered in SVARM after three years, certain issues that deserve further study have been identified. One such issue is co-selection of resistance whereby use of one antimicrobial selects for resistance not only to itself but also to other drugs. Thus, co-selection might explain occurrence of resistance to drugs not currently used, or the persistence of resistance for long periods after the use was discontinued.

Consumption of antimicrobials

Antimicrobials for use in animals in Sweden are only available on veterinary prescription and guidelines emphasising judicious use have been issued. Use for growth promotion was banned in 1986. In year 2002, a total of 17.3 tons of antimicrobials were used for animals in Sweden. This figure is of a similar magnitude as those for 2000 and 2001. The major part (85%) was used for treatment of individual animals. Over the last five years, the overall sales of tetracyclines have decreased markedly. An increase in sales of fluoroquinolones intended for treatment of individual animals (injectables and tablets) was recorded over the last three years. The possible implications of this for resistance in bacteria from animals are, however, difficult to assess as these drugs are authorised for use in several animal species, both food producing animals and pets.

A sudden increase in sales of pleuromutilins was noted between 2001 and 2002. These drugs are used for treatment of swine dysentery and other enteric conditions associated with *Brachyspira* spp. Resistance to pleuromutilins in *Brachyspira* spp. has been reported from other countries. As the therapeutic arsenal available for treatment of these conditions is limited, emergence and spread of resistance to pleuromutilins in *Brachyspira* spp. could have serious consequences for animal health. Therefore, it seems advisable to monitor both use of these drugs and susceptibility of relevant pathogens closely. To minimise the selective pressure, routine use for prevention of disease should be avoided as far as possible.

Clearly, current systems for animal drug statistics need to be refined so that quantities used can be assigned to animal species, and preferably also to different production types. Recently, the Board of Agriculture was appointed as the competent governmental authority in this field, and is to provide such data from 2006. Ideally, it should also be possible to link data on use to animal health records. Such systems would be most valuable to analyse trends in use and resistance, to identify possible risk factors and to assess the degree of compliance with policy recommendations.

Resistance in zoonotic bacteria

Antimicrobial resistance in *Salmonella* from Swedish animals is rare and the situation has been stable since the late 70s, when monitoring of resistance began. The overall prevalence of resistance in each year's material is greatly influenced by the occurrence of multiresistant isolates of *S.* Typhimurium, i.e. resistant to at least three antimicrobials. As these phagetypes (DT104, DT193 and DT120) are rare among food-producing animals, probably a result of the strategies in the Swedish *Salmonella* control programme, the overall prevalence of resistance is low. Nor is there any indication of spread of such clones among the notified incidents in wild animals and pets.

Levels of antimicrobial resistance among C*ampylobacter jejuni* from broiler chickens sampled at slaughter were low and of the same magnitude as year 2001. No isolate was resistant to more than one antimicrobial tested.

Resistance in indicator bacteria

In SVARM, the antimicrobial susceptibility among *Escherichia coli* and *Enterococcus* spp. isolated from healthy animals sampled at slaughter serve as indicator of the selective pressure exerted by antimicrobials used in specific animal populations. Although unlikely to cause disease, these bacteria can constitute a reservoir of transferable resistance genes that can spread to bacteria with potential to cause disease in animals and humans. This year, data on indicator bacteria isolated from broiler chickens is reported.

Overall, the figures for 2002 are low in an international perspective and with few exceptions similar to levels for year 2000 and 2001. Resistance to some antimicrobials can be explained by use of the substance in chicken production, whereas resistance to substances not used might be a consequence of co-selection. Thus, use of one antimicrobial might select for resistance also to other, unrelated, substances. In the combined data for years 2000, 2001 and 2002 there are indications of linked resistance in *E. coli* as well as in enterococci, which implies that co-selection of resistance, might occur.

The proportion of vancomycin resistant enterococci (VRE) in the gastrointestinal tract of broiler chickens is low in Sweden. This concurs with the fact that avoparcin, an antimicrobial feed-

additive selecting for vancomycin resistance, has not been used in Swedish animal production since the early 1980s. The resistance gene (*van*A) is however present among enterococci in broiler chickens as VRE were isolated in selective cultures, with increased sensitivity to detect VRE. Moreover, the apparent prevalence of VRE after selective culture has increased since year 2000. This could partly be the result of improved skills in evaluation of the selective cultures but may also reflect a true increase in prevalence.

Resistance in animal pathogens

Data on antimicrobial susceptibility in animal pathogens, except for udder pathogens, were obtained from the database at SVA. The presented data mostly originate from isolates obtained from diagnostic submissions and might be biased towards treatment failures or otherwise problematic cases. Therefore the results might represent a worst-case scenario and conclusions regarding susceptibility in general must be made with caution.

In pigs, resistance in *E. coli* isolated from diagnostic submissions was more prevalent than among isolates of the same bacterial species from healthy pigs (indicator bacteria). Resistance to tetracycline, streptomycin or the combination trimethoprim-sulphonamide was common (>20%) and have been dominant in the material over the last ten years. Notably, there appears to be an increase in ampicillin resistance over the last years.

Among *Brachyspira hyodysenteriae*, tylosin resistance was common but no resistance to tiamulin was detected. In other countries a progressively decreased susceptibility to tiamulin among *B. hyodysenteriae* isolates have been observed. This emphasises that special attention should be paid to emergence of isolates with decreased susceptibility to tiamulin, especially as the therapeutic arsenal available to treat infections with *B. hyodysenteriae* is limited to few antimicrobials. This aspect is accentuated considering the increase in use of the pleuromutilins in Sweden between 2001-2002.

In *E. coli* from the gastro-intestinal tract of cattle, levels of resistance against streptomycin, tetracycline, ampicillin, the combination trimethoprim-sulphonamide, enrofloxacin and chloramphenicol were high and multiresistance was common. Levels of resistance were conspicuously higher than in *E. coli* isolated from healthy animals year 2000. Among gram-positive udder pathogens (*Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*) isolated from cases of acute mastitis in dairy cows antimicrobial resistance was rare. The most prevalent trait was penicillin resistance due to ß-lactamase production among *S. aureus* (7%). Moreover, one isolate of *S. aureus* was resistant to penicillin and macrolides-lincosamides. This is the first confirmed isolate with this resistance phenotype in Sweden. The finding emphasises the need for bacteriological diagnosis and subsequent susceptibility testing of isolates in mastitis therapy and prophylaxis in order to counteract spread of such clones within and between dairy herds.

As shown in SVARM 2000 and 2001, resistance to the combination trimethoprim-sulphonamide in *Streptococcus zooepidemicus* from the respiratory tract of horses has increased markedly over the last ten years. The figure for year 2002 (28%) is lower than in 1998-99 and 2000 when about half of the isolates were resistant to this drug combination. Whether this is a true decline in occurrence of resistance is uncertain. All isolates were susceptible to penicillin. Among *E. coli* from the genital tract of mares, resistance to trimethoprim-sulphonamide, ampicillin or streptomycin was relatively common.

In dogs, *Staphylococcus intermedius,* isolated from bacteriological samples from skin, were to a large extent ß-lactamase producers (78%) and consequently resistant to penicillin. Resistance to macrolides, lincosamides or tetracycline was also common emphasising the need for culture and subsequent susceptibility testing for an effective therapeutic choice. The need for susceptibility testing also applies to *E. coli* from the urinary tract of dogs and cats. A relatively large proportion (10-20%) of these isolates were resistant to ampicillin, streptomycin, tetracycline or the combination trimethoprim-sulphonamide and multiresistance was not uncommon. Notably, resistance to enrofloxacin was high (15%) in isolates from cats.

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Sammanfattning

Resultaten i denna tredje rapport från SVARM stämmer väl med de från tidigare år, och med andra svenska studier avseende antibiotikaresistens hos bakterier från djur. Rapporten bekräftar att läget är stabilt. Resistens förekommer, men nivåerna är i ett internationellt perspektiv låga. För att bevara det gynnsamma läget är det viktigt att vidmakthålla den goda traditionen av omdömesgillt bruk av antibiotika hos djur, och det goda djurhälsoläget.

Den information om både användning av, och resistens mot, antibiotika som sammanställs i program som SVARM bidrar till att öka förståelsen av resistensepidemiologi. Vissa frågeställningar bör studeras ytterligare. Ett exempel på en sådan frågeställning är co-selektion av resistens. Co-selektion är när användning av ett antibiotikum gynnar (selekterar för) förekomst av resistens inte bara mot just det medlet, utan också samtidigt gynnar förekomst av resistens mot andra antibiotika. Co-selektion kan förklara förekomst av resistens mot antibiotika som inte används, eller kvardröjande av resistens mot medel som inte använts på mycket lång tid.

Användning av antibiotika

I Sverige får antibiotika användas till djur endast när en veterinär har skrivit ett recept. Riktlinjer för förskrivning av antibiotika har utarbetats och där betonas vikten av omdömesgillt bruk. Användningen av antibiotika i tillväxtbefrämjande syfte förbjöds 1986. Den totala förbrukningen var under år 2002 17.3 ton aktiv substans, vilket är ungefär lika mycket som år 2000 och 2001. Merparten (85 %) används för behandling av enskilda djur. Under de senaste fem åren har försäljningen av tetracykliner minskat påtagligt. Användningen av fluorokinoloner för behandling av enskilda djur (tabletter och injektionsmedel) har ökat under de tre senaste åren. Det är svårt att avgöra vad detta har för betydelse för resistensläget, eftersom dessa läkemedel används både till olika typer av livsmedelsproducerande djur och till sällskapsdjur.

Försäljningen av pleuromutiliner har ökat år 2002 jämfört med år 2001. Dessa läkemedel används för behandling av svindysenteri och andra tarmsjukdomar hos gris som förknippas med *Brachyspira* spp. Resistens mot pleuromutiliner hos *Brachyspira* spp har rapporterats från andra länder. Eftersom få medel för behandling av dessa sjukdomar finns tillgängliga kan uppkomst och spridning av resistens mot pleuromutiliner få allvarliga konsekvenser för djurhälsan. Det är därför angeläget att både användning av dessa medel och känslighet hos relevanta sjukdomsframkallande bakterier övervakas noga. För att minimera selektionstrycket bör rutinmässig användning i förebyggande syfte undvikas i görligaste mån.

Dagens system för statistik över användning av läkemedel till djur behöver utvecklas. Användningen bör kunna kopplas till olika djurslag, eventuellt också till typ av djurproduktion. Statens jordbruksverk har nyligen utsetts till ansvarig myndighet inom området, och kommer att rapportera statistik uppdelad på olika djurslag från år 2006. Det vore en fördel om tillgängliga data också kunde analyseras i relation till befintlig djurhälsostatistik. Ett sådant system vore av stort värde vid analys av trender i användning och resistens, samt för att följa efterlevnaden av gällande riktlinjer.

Resistens hos zoonotiska bakterier

Resistens mot antibiotika hos *Salmonella* från svenska djur är ovanligt. Läget har varit stabilt sedan slutet av 1970-talet, då övervakning av resistens hos *Salmonella* från djur påbörjades. Förekomst av resistens under enskilda år påverkas i stor utsträckning av om multiresistenta *S.* Typhimurium (resistenta mot tre eller fler antibiotika) förekommer eller inte. Infektion med fagtyper där multiresistens är vanligt (DT104, DT120 och DT193) förekommer sällan hos livsmedelsproducerande djur i Sverige, troligen som ett resultat av strategier i det svenska salmonellakontrollprogrammet. Detta gör i sin tur att resistens hos *Salmonella* sällan förekommer hos livsmedelsproducerande djur. Det finns heller inga tecken på spridning av multiresistenta kloner bland sällskapsdjur eller vilda djur.

Resistens mot antibiotika hos C*ampylobacter jejuni* från slaktkyckling var ovanligt, vilket stämmer med de resultat som redovisades för 2001. Inget isolat var resistent mot mer än ett av undersökta antibiotika.

Resistens hos indikatorbakterier

I SVARM undersöks antibiotikaresistens hos *E. coli* och *Enterococcus* spp från friska djur som provtas i samband med slakt i syfte att spegla effekten av det selektionstryck som användningen av antibiotika i undersökta populationer utgör. Dessa bakterier kan betraktas som indikatorbakterier. De orsakar sällan sjukdom, men de kan bära resistensgener som kan överföras till bakterier med förmåga att orsaka sjukdom hos djur eller människor. Under 2002 har resistens hos indikatorbakterier från slaktkyckling undersökts.

Siffror för 2002 visar att frekvensen resistenta indikatorbakterier är låg jämfört med vad som rapporteras från andra länder. Med få undantag var nivåerna jämförbara med vad som redovisats år 2000 och 2001. Förekomst av vissa typer av resistens kan förklaras genom användning av motsvarande medel inom kycklingproduktionen, medan resistens mot medel som inte används skulle kunna

förklaras av co-selektion. Analys av samtliga data från åren 2000, 2001 och 2002 antyder att kopplad resistens förekommer hos såväl *E. coli* som enterokocker. Detta innebär att co-selektion kan förekomma.

Andelen vancomycinresistenta enterokocker (VRE) i tarmen hos svenska slaktkycklingar är låg. Detta stämmer väl med det faktum att avoparcin, en fodertillsats som selekterar för vancomycinresistens, inte använts sedan tidigt 80-tal. Resistensgenen (*van*A) förekommer dock bland enterokocker hos slaktkyckling, vilket visas av att VRE isolerats vid odling med selektiva, känsligare metoder. Förekomsten av VRE i det undersökta materialet enligt sådan selektiv odling har ökat sedan år 2000. Detta kan delvis vara ett resultat av ökad skicklighet när det gäller att påvisa bakterien, men kan också vara en sann ökning.

Resistens hos sjukdomsframkallande bakterier från djur

Uppgifter om sjukdomsframkallande bakteriers antibiotikakänslighet har, med undantag för bakterier från juverinflammationer, hämtats från SVAs databas. Data härrör mestadels från isolat från prover som skickats till SVA för rutindiagnostik. Detta innebär att urvalet kan vara vinklat mot särkilt svårbehandlade eller på annat sätt problematiska fall. Generella tolkningar avseende känslighet för antibiotika hos undersökta bakterier bör därför göras med stor försiktighet.

Hos *E. coli* från grisar var resistens vanligare i material från diagnostiska prover än från friska grisar vid slakt (indikatorbakterier). Resistens mot tetracyklin, streptomycin eller kombinationen trimetoprim-sulfa var vanligt förekommande (>20 %) och har dominerat i materialet under det senaste decenniet. Anmärkningsvärt är att resistens mot ampicillin har ökat de senaste åren.

Hos *Brachyspira hyodysenteriae* var resistens mot tylosin vanligt, men ingen resistens mot tiamulin kunde påvisas. I andra länder har känsligheten för tiamulin hos *B. hyodysenteriae* minskat gradvis. Få läkemedel kan idag framgångsrikt användas för behandling av svindysenteri, och det är därför angeläget att uppträdande i Sverige av bakterietammar med sänkt känslighet mot tiamulin uppmärksammas i ett tidigt skede. Betydelsen av att området följs noga understryks av den tidigare nämnda ökningen av användningen av pleuromutiliner från 2001 och 2002.

Hos *E. coli* från tarmkanalen hos nötkreatur var andelen resistens mot streptomycin, tetracyklin, ampicillin, kombinationen trimetoprim-sulfa, enrofloxacin och kloramfenikol hög, och multiresistens var vanligt. Andelen resistens var påtagligt högre än bland bakterier från friska djur år 2000. Bland gram-positiva bakterier (*Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*) isolerade från akuta juverinflammationer hos mjölkkor var antibiotikaresistens ovanlig. Mest förekommande var resistens mot penicillin orsakad av ß-laktamasproduktion hos *S. aureus* (7 %). Ett isolat var resistent både mot penicillin, makrolider och linkosamider. Detta är det första konfirmerade isolatet i Sverige med denna fenotyp. Fyndet understryker behovet av att vid behandling ha en bakteriologisk diagnos och resistensbestämning, så att spridning av sådana stammar inom och mellan besättningar kan motverkas.

I SVARM 2000 och 2001 visades att andelen resistens mot trimetoprim-sulfa hos *Streptococcus zooepidemicus* från luftvägar hos häst ökat under den senaste tio åren. Motsvarande siffra för 2002 var lägre (28 %) än för 1998-99 och 2000, då ungefär hälften av isolaten var resistenta mot denna kombination. Det är oklart om detta är en sann minskning eller inte. Liksom tidigare var alla isolat känsliga för penicillin. Bland *E. coli* från könsorgan hos ston var resistens mot trimetoprim-sulfa, ampicillin eller streptomycin relativt vanlig.

Hos hund var *Staphylococcus intermedius* isolerade från hudprover i stor utsträckning (78 %) ß -laktamas bildande, och alltså resistenta mot penicillin. Resistens mot makrolider, linkosamider eller tetracyklin var också vanligt, vilket understryker behovet av odling och resistenbestämning som underlag för val av behandling. Detta gäller också för *E. coli* från urinvägar hos hund och katt. En relativt stor andel (10-20 %) av isolat från urinvägar hos dessa djurslag var resistenta mot ampicillin, streptomycin, tetracyklin eller kombinationen trimetoprim-sulfa, och multiresistens var inte ovanligt. Anmärkningsvärt var att andelen resistens mot fluorokinoloner hos *E. coli* från katter var hög (15 %).

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Use of antimicrobials

Through an initiative of SVA and Apoteket AB (the National Corporation of Swedish Pharmacies), statistics on total sales of antibiotics for use in animals in Sweden are available since 1980. For a review of the figures from 1980-2000 as well as references to publications on which that review is based, see SVARM 2000. Up to and including the year 2002, data presented are sales from wholesalers to pharmacies. From 2003, the basis for the statistics will be changed to sales from pharmacies.

Material included

In Sweden, antimicrobials for use in animals are only available on veterinary prescription and all pharmaceuticals are dispensed by pharmacies. In 1986, the Feedstuffs Act restricted the use of antibiotics for veterinary medicinal purposes, i.e. their use as growth promoters was banned.

Drug statistics are based on sales figures provided by Apoteket AB and represent the total amount of antimicrobials authorised for veterinary use sold from wholesalers to pharmacies calculated to kg active substance. These figures include antimicrobial formulations for systemic, intramammary and obstetric use, and intestinal anti-infectives, for all animal species (food producing animals, fish, pets and horses etc). It is assumed that the amount sold is also used during the observation period. Drugs authorised for human use but prescribed for animals are not included. Such drugs are primarily prescribed in small animal medicine and their use is declining as the number of products authorised for veterinary use is increasing.

Details on animal numbers are found in Appendix 1 and on methodology in Appendix 2.

Use of antibiotics - the figures for 2002

Overall use

The total usage of antimicrobials is presented in table AC I. The different substances are not equal in their biological activity per weight unit and therefore, each substance group should be evaluated separately. Nonetheless, the total figures indicate trends in the material. The overall use has decreased since the mid 90s, but over the last three years it is roughly unchanged. The use of tetracyclines has decreased by half since 1998. The figures for this class of antimicrobials from the three last years include drugs marketed with special licence.

Between 2001 and 2002, the use of cephalosporins and pleuromutilins increased. For the cephalosporins, an increasing trend has been noted since 1997, when drugs of this class were introduced on the Swedish market for use in pets. Mostly, the trend reflects an increased prescription to pets of drugs authorised for veterinary use instead of off-label prescription of drugs of the same class authorised for humans. As drugs authorised for humans are not included in the statistics, the total use of cephalosporins may well be unchanged. As to the pleuromutilins, the use decreased between 1998 and 1999, but the figures for 2002 are back to the same order of magnitude as those of 1998. The pleuromutilins are used exclusively in pigs with swine dysentery as the main indication. Between 1998 and 2002, the number of slaughter pigs has decreased by 15%. Thus, the incidence of use has increased.

In chickens, ionophoric antibiotics are given to control coccidiosis. The sales of these products are discussed under the section on group treatment (see Table AC III).

Table AC I. Yearly sales of antimicrobial drugs authorised for veterinary use expressed as kg active substance (sales statistics from Apoteket AB)

ATCvet	Substance class	1980	1988	1996	1998	1999	2000	2001	2002
QG01AA, QJ01A	Tetracyclines	9 819	4 691	2 698	2 897	2 251	1 754[4]	1 453[4]	1 415[4]
QJ01B	Chloramphenicol	47	35	-	-	-	-	-	-
QJ01CE, QJ01R, QJ51	Penicillin G and V[1]	3 222	7 143	8 818	8 547	8 692	8 254	8 414	8 179
QJ01CA, QJ01CR	Aminopenicillins	60	655	835	824	809	852	752	767
QJ01D	Other beta-lactams	9	-	-	133	245	315	474	676
QA07AA, QJ01G, QJ01R, QJ51R	Aminoglycosides	5 274	3 194	1 164	930	846	797[4]	770[4]	753[4]
QA07AB, QJ01E	Sulphonamides	6 600	3 072	2 198	2 345	2 403	2 338	2 485	2 477
QJ01E	Trimethoprim & derivatives	134	250	339	390	397	390	414	414
QJ01F	Macrolides & lincosamides	603	1 205	1 649	1 846	1 467	1 352	1 510	1 412
QJ01MA	Fluoroquinolones			173	175	155	156	182	185
QJ01XX92, QJ01XX94	Pleuromutilins		124	1 142	1 032	847	871	841	988
QJ01MB	Quinoxalines	6 250	7 164	1 098	-	-	-	-	-
QJ01XX91	Streptogramins	-	1 088	525	150	125	-	-	-
	Other substances[2]	861	1 567	-	-	-	-	-	-
	Antimicrobial feed additives[3]	8 380	-	-	-	-	-	-	-
Total		**41 259**	**30 189**	**20 639**	**19 269**	**18 237**	**17 079**	**17 295**	**17 266**

[1] Calculated as benzyl-penicillin; [2] Mainly nitroimidazoles, QP51AA; [3] Substances included are avoparcin, bacitracin, nitrovin, oleandromycin and

[4] Drugs marketed with special licence are included.

Use for systemic treatment of individual animals

In table AC II, the volume sold in form of products formulated for use in individual animals, excluding topical, intrauterine and intramammary use is presented. The use of most groups has decreased or been relatively unchanged over the last five years. A large part of the injectables is probably used for treatment of bovine mastitis. Therefore, much of the decrease may be explained by a steadily decreasing number of dairy cows. It should be noted, however, that many of the drugs of concern are also used in horses. Annual changes in the number of horses (increases or decreases) can therefore have an influence on the figures. Currently, there are no reliable figures on number of horses so any interpretation of trends in sales of drugs of this category must be made with great caution. The increased sale of cephalosporins is related to use in pets and was commented above. The use of fluoroquinolones for individual treatment has increased by 18% over the last five years.

Use for treatment of groups or flocks

Of special interest when considering the risk for development of resistance is the consumption of antimicrobials intended for group or flock medication. Of the total sales of antimicrobials for animals, the proportion of drugs authorised for treatment of groups of animals via feed or water has decreased steadily over the years (Table AC III). Only four classes of antimicrobials of this type remain on the market. All groups except the pleuromutilins show a declining trend since at least the mid 90s. Pleuromutilins (tiamulin, valnemulin) are only authorised for use in pigs, with swine dysentery as the main indication. The sudden increase in use between 2001 and 2002 could be explained either by a general increase in incidence of swine dysentery, or by use of these drugs for preventive purposes in one or several larger production units.

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

Units of measurement for group treatment of pigs

For estimates of treatment incidence, data must be broken down at least by animal species. Unfortunately, the possibilities to do so using current systems are limited as many antimicrobials are authorised for use in several animal species. However, on basis of knowledge on how these drugs are or have been used, a selection of products that have only or mostly been used for treatment of groups of pigs can be made. Before 1986, antimicrobial growth promoters were used both for chickens and pigs. For the latter, the quinoxalines (olaquindox, carbadox) were by far the most widely used. Group treatment of calves is not common

Table AC II. Yearly sales of antimicrobial drugs authorised for indivual treatment expressed in kg acitve substance. Intramammaries (QJ51) and formulations for dermatological use (QD06), as well as local treatment of the genito-urinary tract (QG01) are not included (sales statictics from Apoteket AB)

ATCvet	Substance class	1980	1988	1996	1998	1999	2000	2001	2002
QA07A	Intestinal anti-infectives	NA[3]	NA[3]	863	649	607	587[5]	614[5]	584[5]
QJ01A	Tetracyclines	549	514	596	656	695	634	623	628
QJ01B	Chloramphenicol	47	35	-	-	-	-	-	-
QJ01C	Penicillins[1,2]	3 222	7 143	9 560	9 287	9 424	9 037	9 095	8 894
QJ01D	Cephalosporins	-	-	-	133	245	315	474	676
QJ01E	Sulphonamides & trimethoprim	6 734[4]	3 322[4]	2 033	2 335	2 376	2 336	2 478	2 483
QJ01F	Macrolides & lincosamides	295	454	675	645	559	531	522	477
QJ01G	Aminoglycosides[2]	5274[4]	3194[4]	650	535	528	474	454	460
QJ01M	Fluoroquinolones	-	-	147	150	144	150	169	178
QJ01X	Pleuromutilins	-	23	73	64	52	56	48	49

[1] Procaine-penicillin calculated as benzyl-penicillin; [2] The amount includes QJ01R, combinations; [3] Separate figures not available for 1980 and 1988, for these years the intestinal anti-infectives are included in the sulphonamides and aminoglycosides; [4] Figures include intestinal anti-infectives (QA07A); [5] Drugs marketed with special licence are included.

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

ATCvet	Substance class	1980	1988	1996	1998	1999	2000	2001	2002
QJ01A	Tetracyclines	9 270	4 177	2 089	2 230	1 545	1 111[3]	822[3]	777[3]
QJ01F	Macrolides & lincosamides	308	751	975	1 201	908	821	988	935
QJ01M	Fluoroquinolones	-	-	27	25	11	7	13	7
QJ01X	Pleuromutilins	-	101	1069	969	795	815	793	939
QP51A	Nitroimidazoles	791	1 557	-	-	-	-	-	-
QJ01M	Quinoxalines	6 250	7 164	1 098	-	-	-	-	-
QJ01X	Streptogramins	-	1 088	525	150	125	-	-	-
-	Antibacterial feed additives[1]	8 380	700	-	-	-	-	-	-
QP51AH	Ionophoric antibiotics (coccidiostats)	390	6 991	11 643	9 941	9 562[2]	9 368[2]	10 019[2]	8 439[2]

[1] Substances included are avoparcin, bacitracin, nitrovin, oleandromycin and spiramycin; [2] From 1999 regulated and classified as feed additives (dir 70/524/EEC). Figures from 1999 and onwards are from the Feed Control of the Board of Agriculture (www.sjv.se); [3] Drugs marketed with special licence are included.

practice in Sweden. Very small amounts are used for poultry (SVARM 2000). Hence, it can be assumed that the bulk of the sales of drugs for group treatment from 1988 and onwards was intended for treatment of enteric and respiratory infections of pigs. In the upper part of Table AC IV, figures on total sales in kg of products mostly or exclusively intended for treatments of pigs through medication in feed or water are shown. The total use measured as kg active substance has decreased markedly over the years, e.g. with 42% from 1998 to 2002.

When interpreting the data, it is important to bear in mind that the numbers of pigs has mostly been 5-10% lower than in the early 80s. Therefore, sales figures corrected for population size might give a better estimate of trends in use. Figures on amount sold of each class of antimicrobial in g/pig are presented in the mid section of Table AC IV. Also with this measure, a decrease in use from 1998 to 2002 is recorded (31%), but it is slightly less than when measured as uncorrected kg.

The recommended dose for the various substances varies widely between substances. In order to get a better measure of the exposure of the population, a defined dose can be used to calculate the amount of feed that could be mixed with the amount of active substance sold. The doses shown in the lower part of Table AC IV are mostly those suggested by Beskow (cited and used in Wierup *et al*, 1987) or the authorised dose as given in the Swedish Compendium of Veterinary Drugs (FASS VET., 2002). A measure based on defined doses has the merit of correcting for differences in potency. If divided by the number of pigs slaughtered, it will also account for variations in the number of pigs produced. The calculated total sum of kg medicated feed/pig is shown in Table AC IV. With this measure, a decrease from 1998 to 2002 is noted (8%), but it is less pronounced than for the other two measures.

To summarise, a decrease of use over time is noted for all three units of measurement, but the pattern and magnitude of the decrease is different depending on the unit used (Figure AC I).

Table AC IV. Yearly sales of antimicrobials mostly or exclusively intented for treatment of pigs through feed or water (group medications), expressed in different units of measurment. Based on sale statistics from Apoteket AB and Statistics Sweden

Substance class	**Dose (ppm)**	**1980**	**1988**	**1996**	**1998**	**1999**	**2000**	**2001**	**2002**
Amount in kg									
Tetracyclines[1]		9 270	4 177	2 089	2 230	1 545	1 111	822	777
Macrolides & lincosamides		308	751	975	1 201	908	821	988	935
Fluoroquinolones		-	-	27	25	11	7	13	7
Quinoxalines		6 250	7 164	1 098	-	-	-	-	-
Streptogramins[2]		-	1 088	525	150	125	-	-	-
Pleuromutilins		-	101	1069	969	795	815	793	939
Nitroimidazoles		791	1 557	-	-	-	-	-	-
Total		*16 619*	*14 838*	*5 783*	*4 575*	*3 384*	*2 754*	*2 616*	*2 658*
Amount in g/pig									
Tetracyclines[1]		2.23	1.13	0.54	0.58	0.41	0.34	0.26	0.24
Macrolides & lincosamides		0.07	0.20	0.25	0.31	0.24	0.25	0.31	0.28
Fluoroquinolones		-	-	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Quinoxalines		1.50	1.93	0.29	-	-	-	-	-
Streptogramins[2]		-	0.29	0.14	0.04	0.03	-	-	-
Pleuromutilins		-	0.03	0.28	0.25	0.24	0.25	0.25	0.29
Nitroimidazoles		0.19	0.42	-	-	-	-	-	-
Total		*4.00*	*4.00*	*1.50*	*1.18*	*0.92*	*0.85*	*0.82*	*0.81*
Calculated kg feed/pig									
Tetracyclines[1]	*1000*	2.23	1.13	0.54	0.58	0.41	0.34	0.26	0.24
Macrolides & lincosamides	*100*	0.74	2.02	2.54	3.10	2.39	2.53	3.09	2.85
Fluoroquinolones	*50*	-	-	0.14	0.13	0.06	0.04	0.08	0.04
Quinoxalines	*50-160*[3]	30.10	12.07	1.79	-	-	-	-	-

[1] 1980-1984 only quantities mixed in feed at feed mills (Wierup *et al* 1989); [2] Excluded before 1988 as before that time, most of the sales were for use in poultry;

[3] Before 1986 50 ppm and from 1986 160 ppm.

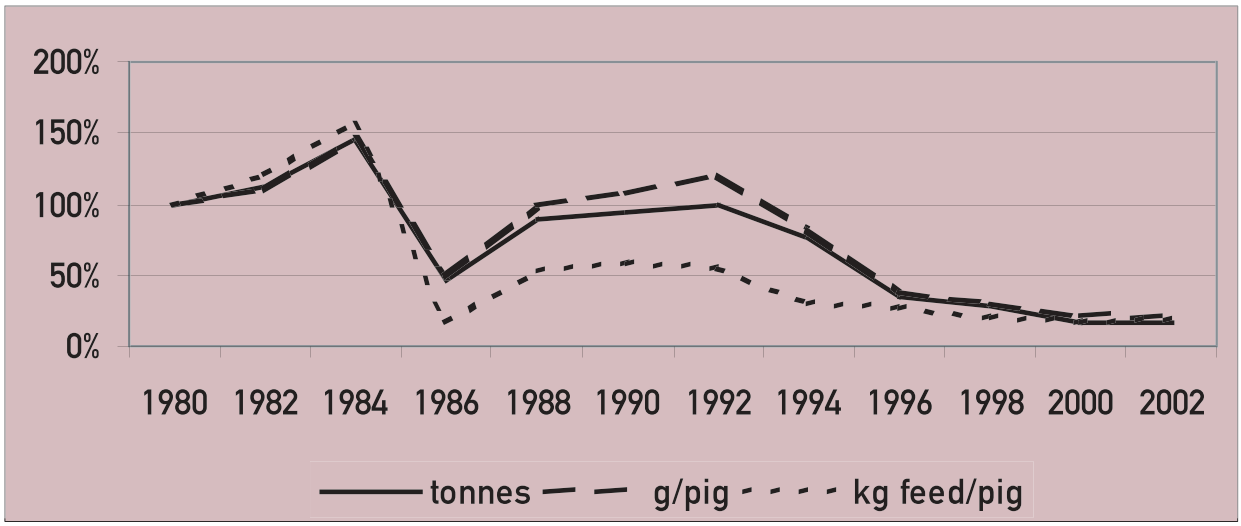

Figure AC I. Total sales of formulations of antimicrobials mostly or exclusively intended for treatmeant of pigs through feed or water (group medications), expressed in different units of measurment. Based on statistics from Apoteket AB and Statistics Sweden.

General comments

Overall, no dramatic changes in use of antimicrobials were noted when figures from the years 2001 and 2002 were compared. Over the last five years, the overall use of antimicrobials intended for treatment of groups or flocks of animals has decreased while the amount of drugs for treatment of individual animals has remained relatively unchanged. The sales of tetracyclines have decreased markedly over the last five years, while sales of fluoroquinolones and pleuromutilins have increased.

The increase in sales of fluoroquinolones derives from sales of products intended for treatment of individual animals (injectables and tablets). The total figures in kg may appear low compared with other drug classes, but fluoroquinolones have a high inherent activity and are given in low doses, so even small changes may represent a biologically significant increase in number of animals treated. The possible implications of this recorded increase for resistance in bacteria from animals are, however, difficult to assess as these drugs are authorised for use in several animal species, both food producing animals and pets.

A sudden increase in sales of pleuromutilins was noted between 2001 and 2002. These drugs are used for treatment of swine dysentery and other enteric conditions associated with *Brachyspira* spp. Resistance to pleuromutilins in *Brachyspira* spp. has been reported from other countries. As the therapeutic arsenal available for treatment of these conditions is limited, emergence and spread of resistance to pleuromutilins in *Brachyspira* spp. could have serious consequences for animal health. Therefore, is seems advisable to monitor both use of these drugs and susceptibility of relevant pathogens closely. To minimise the selective pressure, routine use for prevention of disease should be avoided as far as possible.

For more precise estimates of treatment incidence, data must be broken down at least by animal species. Unfortunately, the possibilities to do so using current systems are limited. At most, estimates, or 'guesstimates', can be made for specific drug categories based on knowledge of how the drugs are or have been used. The possibility to assign subsets of the use to specific species or categories of animals is a prerequisite for development of more informative units of measurement. When a subset of drugs used mostly or exclusively in pigs was examined by use of different units, an overall decrease in use was recorded for all units. The magnitude of this decrease was, however, considerably less pronounced when a unit that corrects both differences in potency of the drugs and population size (kg feed/pig) was used (Figure AC I). This emphasises the importance of development of standard units of measurement of animal drugs. Preferably, such units should be agreed upon on an international level. To this end, on initiative of the WHO collaborating centre for drug statistics, an international *ad hoc* group with the remit to assign defined doses for animals has been formed.

Sweden has a long tradition of monitoring use of antimicrobials for animals. Data are followed closely by the stakeholders (e.g. experts, decision makers, practising veterinarians and farmers organisations) and are often subject of debate. Current systems need to be refined so that quantities used can be assigned to animal species, and preferably to different production types. Ideally, it should also be possible to link data on use to animal health records. Such systems are needed to analyse trends in use and resistance, to identify possible risk factors and to assess the degree of compliance with policy recommendations.

The monitoring program encompasses zoonotic bacteria isolated from animals of Swedish origin. This year data on antimicrobial susceptibility among *Salmonella enterica* and among *Campylobacter jejuni* and hippurate-negative thermophilic *Campylobacter* spp. are presented. Information on the zoonotic aspects of these infections in Sweden is available in Zoonoses in Sweden (2001).

It is to be observed that some breakpoints for resistance used in previous SVARM-reports have been changed. To facilitate comparisons when data from previous years are presented, levels of resistance have been recalculated using the current breakpoints. For a summary of breakpoints used see Appendix 3.

Salmonella

Isolates included

Any finding of *Salmonella* in animals is notifiable in Sweden and confirmation at SVA of at least one isolate from each incident is mandatory. From these isolates, one from each animal species (warm-blooded wild and domesticated) involved in each notified incident year 2002 are included in the material.

In Sweden, monitoring of antimicrobial susceptibility among *Salmonella* of animal origin has been performed regularly since 1978. Although the antimicrobials included in the test panels have varied, microdilution methods have been used in all these surveys. For comparison, data from previous years are therefore presented together with data for 2002.

Results and comments

A total of 51 isolates are included in the material (Table S I). Of these, 31 were *S.* Typhimurium, four *S.* Dublin and 16 isolates were other serovars. The majority of isolates (46%) emanated from major food producing animals and the remainder from pets and horses (30%) and wild animals (25%) (Table S I). One isolate of *S.* Dublin from cattle and one isolate of *S.* species from a cat were not available for susceptibility testing. The distributions of MICs for the remaining 49 isolates are given in Table S IIA and S IIB.

Only three isolates (6%) were classified as resistant to any of the antimicrobials tested. One of these, S Typhimurium DT 40 isolated from a cat, was resistant to nalidixic acid. The other two resistant isolates, *S.* Schleissheim from a wild bird and *S.* Agona from a dog, were resistant to streptomycin and to streptomycin and sulphonamides, respectively. The latter was the only isolate resistant to more than one antimicrobial.

The low level of resistance among *Salmonella enterica*, as well as in the subset *S.* Typhimurium, year 2002 agrees with the results for previous years (SVARM 2000 and 2001). Further, among *S.* Typhimurium, levels of resistance have been stable, the only apparent trend is a lower level of resistance to streptomycin since 1999 compared to the preceding period (Table S. III).

It is apparent that the occurrence of multiresistant isolates, i.e. resistant to at least three antimicrobials, in each year's material greatly influences the overall prevalence of resistance. Among *S.* Typhimurium, five isolates were multiresistant in 1999 and two isolates in each of the years 2000 and 2001. These isolates were of the phage types DT 104, DT 193 or DT 120. The impact on the overall levels of resistance each year can be appreciated from Table S III. The material from 2002 did not include any multiresistant isolate and hence only resistance to a single antimicrobial (nalidixic acid) occurred in one isolate.

The material in the years 1997 to 2002 consists of one isolate from each notified incident of *Salmonella* in Sweden, including those detected in food-producing animals in the *Salmonella* control programme. From a public health perspective, the prevalence of resistance in *Salmonella* from food-producing animals is of greater importance than resistance in isolates from wild animals or pets. Therefore a subset of the 218 isolates from food-producing animals years 1997-2002 is presented in Table S IV. The overall prevalence of resistance is low also in this subset. In the whole material only 18 isolates (8%) were resistant to any of the antimicrobials tested and five isolates (2%) were multiresistant. All multiresistant isolates were *S.* Typhimurium, two each of the phage-types DT104 and DT193 and one isolate of DT120. These isolates were resistant to ampicillin, streptomycin, tetracycline and sulphonamides. In addition, the DT104 and DT120 isolates were resistant to chloramphenicol.

In the light of this, the overall situation of antimicrobial resistance in *Salmonella* is most favourable. There is no evident spread of multiresistant clones among food-producing animals within the country, probably as a result of the strategies in the Swedish *Salmonella* control programme. Nor is there among the notified incidents in wild animals any indication of spread of such clones as only one of the 70 *Salmonella enterica* isolates tested since 1997 was multiresistant.

Table S I. Number of isolates of *Salmonella enterica* included in 2002 presented by serotype and source of isolates

Serotype	Phage type	Cattle	Pig	Poultry	Dog	Cat	Wild bird	Total
S. Typhimurium	120	1						**1**
	195						1	**1**
	40		1			8	1	**10**
	41						2	**2**
	NST	2	2	1		1	4	**10**
	1	1	1					**2**
	U277					2	1	**3**
	Not typed		1				1	**2**
S. Agona					1			**1**
S. Braenderup							1	**1**
S. Dublin		4						**4**
S. Enteritidis		1		2				**3**
S. Livingstone				1				**1**
S. Mbandaka			1					**1**
S. Poona					1			**1**
S. Rissen				1				**1**
S. Saint-Paul				1				**1**
S. Schleissheim							1	**1**
S. Senftenberg							1	**1**
S. subspecies I		1			1			**2**
S. subspecies II				1				**1**
S. species						1		**1**
Total		**10**	**6**	**7**	**3**	**12**	**13**	**51**
Percent of total		**20%**	**12%**	**14%**	**6%**	**24%**	**25%**	

Table S II. Distribution of MICs for all *Salmonella* enterica (A) (n=49) and for the subset *Salmonella* Typhimurium (B) (n=31) from animals in 2002. Bold vertical lines indicate breakpoint for resistance

A *Salmonella enterica*	**Percent resistant**	**Distribution (%) of MICs[1] (mg/L)**															
Substance		**≤ 0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**>512**
Amoxi/Clavulan[2]	0						26.5	69.4	4.1								
Ampicillin	0						77.6	22.4									
Apramycin	0						2.0			63.3	32.7	2.0					
Ceftiofur	0				2.0	22.4	69.4	6.1									
Chloramphenicol	0							20.4	71.4	8.2							
Enrofloxacin	0		28.6	71.4													
Florfenicol	0							10.2	75.5	14.3							
Gentamicin	0					2.0	63.3	30.6	4.1								
Nalidixic acid	2							2.0	91.8	4.1			2.0				
Neomycin	0							61.2	36.7	2.0							
Streptomycin	4									8.2	44.9	42.9	2.0			2.0	
Sulphamethoxazole	2													81.3	16.7		2.1
Tetracycline	0					2.0		65.3	32.7								
Trimethoprim	0				28.6	67.3	4.1										

B *Salmonella* Typhimurium	**Percent resistant**	**Distribution (%) of MICs[1] (mg/L)**															
Substance		**≤ 0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**>512**
Amoxi/Clavulan[2]	0						29.0	64.5	6.5								
Ampicillin	0						80.6	19.4									
Apramycin	0						3.2			71.0	25.8						
Ceftiofur	0					25.8	64.5	9.7									
Chloramphenicol	0							25.8	74.2								
Enrofloxacin	0		29.0	71.0													
Florfenicol	0							16.1	83.9								
Gentamicin	0					3.2	61.3	35.5									
Nalidixic acid	3								93.5	3.2			3.2				
Neomycin	0							64.5	32.3	3.2							
Streptomycin	0									3.2	54.8	41.9					
Sulphamethoxazole	0													77.4	22.6		
Tetracycline	0					3.2		64.5	32.3								
Trimethoprim	0				29.0	64.5	6.5										

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2/1.

Table S III. Occurrence of resistance to antimicrobials and source of isolates in *Salmonella* Typhimurium from animals 1978 to 2002

Substance	Breakpoint resistance (mg/L)	Percent resistance								
		1978-86 (n=117)	1987-88[1, 2] (n=8)	1989-92 (n=79)	1993-96 (n=87)	1997-98 (n=50)	1999 (n=101)	2000 (n=46)	2001 (n=31)	2002 (n=31)
Amoxicillin/Clavulanic acid	>8/4	-	-	-	-	-	-	2	6	0
Ampicillin	>8	2	0	3	8	12	5	2	6	0
Apramycin	>32	-	-	-	-	-	-	0	0	0
Ceftiofur	>2	-	-	-	-	-	-	0	0	0
Cephalothin	>16	-	-	1	0	0	3	-	-	-
Chloramphenicol	>16	4[3]	0[3]	3[3]	6[3]	12[3]	2[3]	2[3]	6[3]	0
Enrofloxacin	>0.25	-	-	<1	1	0	1	0	0	0
Florfenicol	>16	-	-	-	-	-	-	2	6	0
Gentamicin	>8	-	-	0	0	0	0	0	0	0
Nalidixic acid	>16	-	-	-	-	-	-	4	3	3
Neomycin	>8	0	0	4	0	2	0	0	3	0
Streptomycin	>32	78	12	25	13	20	6	4	6	0
Tetracycline	>8	14	0	3	7	12	5	2	6	0
Trimethoprim	>8	-	-	-	-	-	-	0	0	0
Trimethoprim/Sulphamethoxazole	>0.5/9.5	0	0	1	1	8	3	-	-	-
Percent of isolates from:										
Cattle, sheep, pigs, poultry		100	100	59	55	56	23	57	39	36
Horse, cats, dogs		-	-	15	22	16	53	37	38	32
Wildlife		-	-	26	23	28	24	7	23	32

[1] Only isolates from cattle; [2] 1988 includes isolates to September, isolates from October-December 1988 given under 1989; [3] Breakpoint for resistance >8 mg/L.

Table S IV. Distribution of MICs for all *Salmonella enterica* (A) (n=218) and for the subset *Salmonella* Typhimurium (B) (n=99) from food-producing animals years 1997-2002. Bold vertical lines indicate breakpoint for resistance

A *Salmonella enterica*	Percent resistant	Distribution (%) of MICs[1] (mg/L)															
Substance		**≤ 0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**>512**
Amoxi/Clavulan[2]	0[3]						68.1	31.9									
Ampicillin	2					3.2	64.2	29.4	0.9			2.3					
Apramycin	0[3]							1.1	14.3	53.8	28.6	2.2					
Ceftiofur	0[3]				7.7	36.3	53.8	2.2									
Chloramphenicol	1							19.3	68.8	10.6		1.4					
Enrofloxacin	<1				99.5		0.5										
Florfenicol	0[3]							15.4	70.3	14.3							
Gentamicin	0						53.7	16.1	30.3								
Nalidixic acid	1[3]								35.2	42.9	20.9	1.1					
Neomycin	0						9.6	59.2	29.8	1.4							
Streptomycin	7							0.5	1.8	14.7	38.5	37.6	3.7	2.3	0.9		
Sulphamethoxazole	1[3]												46.2	48.4	4.4		1.1
Tetracycline	2						14.2	55.0	26.6	1.8			0.9	1.4			
Trimethoprim	0[3]				17.6	69.2	12.1		1.1								

B *Salmonella* Typhimurium	Percent resistant	Distribution (%) of MICs[1] (mg/L)															
Substance		**≤ 0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**>512**
Amoxi/Clavulan[2]	0[4]						64.6	35.4									
Ampicillin	5					1.0	59.6	33.3	1.0			5.1					
Apramycin	0[4]							2.1	6.3	56.3	35.4						
Ceftiofur	0[4]					37.5	58.3	4.2									
Chloramphenicol	3							18.2	75.8	3.0		3.0					
Enrofloxacin	0				100.0												
Florfenicol	0[4]							12.5	85.4	2.1							
Gentamicin	0						49.5	22.2	28.3								
Nalidixic acid	2[4]								35.4	37.5	25.0	2.1					
Neomycin	0						6.1	65.7	28.3								
Streptomycin	5								1.0	6.1	44.4	43.4		3.0	2.0		
Sulphamethoxazole	0[4]												52.1	41.7	6.3		
Tetracycline	5						7.1	55.6	29.3	3.0			2.0	3.0			
Trimethoprim	0[4]				25.0	58.3	16.7										

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2/1. [3] 91 isolates tested, [4] 48 isolates tested.

Campylobacter from animals

Isolates included

From the Swedish *Campylobacter* control programme year 2002 a total of 100 isolates from broiler chickens were randomly selected for susceptibility testing.

Isolates were identified as *Campylobacter jejuni* or as hippurate-negative thermophilic *Campylobacter*. Antimicrobials included in the test panels and concentration ranges are given in Table Camp I. For details on methodology, including sampling strategy, see Appendix 3.

Results and comments

The majority of isolates were identified as *C. jejuni* (84%) and only 16% were classified as hippurate-negative thermophilic *Campylobacter* spp. As the isolates are obtained within the framework of the Swedish *Campylobacter* control programme, it can be assumed that the material is representative of *Campylobacter* prevalent in broiler chickens in Sweden.

Overall, levels of antimicrobial resistance among *C. jejuni* were low (Table Camp I). No isolate was resistant to more than one antimicrobial tested. Resistance to ampicillin (10%) was the most prevalent trait. One isolate was resistant to tetracycline. Occurrence of resistance differs numerically from year 2001 for the traits ampicillin and nalidixic acid but differences are not statistically significant.

Among the 16 isolates of *C.* spp., one isolate was resistant to nalidixic acid and enrofloxacin and two isolates were resistant to tetracycline or ampicillin, respectively. These levels tally with those in 2001 when one of seven isolates tested was resistant to nalidixic acid and enrofloxacin.

Interestingly, among 1040 clinical isolates of *C. jejuni* from humans in one county of Sweden years 1991-2000 resistance to ciprofloxacin and tetracycline occurred in 31 and 24% of the isolates, respectively, (SWEDRES 2001). Both these resistance traits were rare among isolates from chickens (Table Camp I). The majority of the human isolates however were of foreign origin.

Table Camp I. Occurrence of resistance (%) among isolates of *Campylobacter jejuni*. from chickens, 2002. Data for 2001 (chickens and cattle) are given for comparison (SVARM 2001

Substance	Range tested (mg/L)	Breakpoint resistance (mg/L)	% Resistant		
			Chickens		Cattle
			2002s n=84	2001 n=43	2001 n=67
Ampicillin	0.5-64	>16	10	2	6
Enrofloxacin	0.03-4	>1	0	2	2
Erythromycin	0.12-16	>16	0	0	0
Gentamicin	0.25-8	>8	0	0	0
Nalidixic acid	1-128	>16	0	5	2
Tetracycline	0.25-32	>8	1	0	0

Table Camp II. Distribution of MICs for *Campylobacter jejuni* from chickens (n=84), 2002. Data for 2001 (n=43) are given for comparison (SVARM 2001). Bold vertical lines indicate breakpoint for resistance

Substance	Year	Percent resistant	Distribution (%) of MICs[1] (mg/L)													
			≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Ampicillin	-02	10					7.1	3.6	21.4	44.0	10.7	3.6	4.8	4.8		
	-01	2					2.3	11.6	46.5	30.2	7.0				2.3	
Enrofloxacin	-02	0		27.4	63.1	7.1	2.4									
	-01	2		51.2	44.2			2.3			2.3					
Erythromycin	-02	0				6.0	26.2	47.6	17.9	2.4						
	-01	0			2.3	14.0	62.8	16.3	4.7							
Gentamicin	-02	0					29.8	52.4	17.9							
	-01	0					67.4	27.9	4.7							
Nalidixic acid	-02	0							14.3	51.2	31.0	3.6				
	-01	5							23.3	72.1						4.7
Tetracycline	-02	1				96.4	2.4						1.2			
	-01	0				95.3		2.3	2.3							

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration.

Resistance in indicator bacteria

The prevalence of acquired resistance to antimicrobials among bacteria of the normal enteric microflora can serve as an indicator of the selective pressure exerted by use of antimicrobial agents in exposed populations. Although these bacteria are unlikely to cause diseases, they form a reservoir of transferable resistance determinants from which resistance genes can spread to bacteria responsible for infections in animals or humans. Thus, surveillance of resistance among indicator bacteria in the normal enteric microbiota from healthy animals can be of great value to detect trends and to follow the effects of interventions.

In SVARM, *Escherichia coli* and *Enterococcus* spp. serve as indicator bacteria. The report for year 2002 presents data on isolates from broiler chickens.

Of special interest in monitoring antimicrobial susceptibility among indicator bacteria is the occurrence of specific patterns of resistance. Such patterns, or phenotypes, can indicate that resistance genes are located on the same genetic element. The danger of such elements is evident as a single transfer event conveys resistance to several antimicrobials to the recipient bacterium (co-transfer). Thereby, use of one antimicrobial can select for resistance to other unrelated antimicrobials (co-selection). In SVARM 2002, analyses of associations between resistance to different antimicrobials were performed on the combined data for years 2000, 2001 and 2002. To this end the Chi-Square test was used for statistical inference on the likelihood that isolates resistant to one antimicrobial also were resistant to another. The same test was used for analysis of differences in occurrence of resistance between years 2000, 2001 and 2002.

It is to be observed that some breakpoints for resistance used in previous SVARM-reports have been changed. To facilitate comparisons when data from previous years are presented, levels of resistance have been recalculated using the current breakpoints. For a summary of breakpoints used see Appendix 3.

Isolates included

Escherichia coli and *Enterococcus* spp. were isolated from ceacal content from broiler chickens sampled at slaughter. Each isolate originates from a unique flock but not always from a unique production site. Antimicrobials included in the test panels and concentration ranges used are given in Table EC I and ENT I. For details on methodology, including sampling strategy, see Appendix 3.

Escherichia coli

Results and comments

The material includes 306 isolates of *E. coli* from broiler chickens. Isolates were obtained from 87% of 351 samples cultured, a similar isolation frequency as in SVARM 2000 and 2001.

The majority of isolates (80%) were sensitive to all fourteen antimicrobials tested but 61 isolates were resistant to at least one substance. Sulphonamide resistance was the most common trait (10%) (Table EC I and EC IV). Resistance against ampicillin, nalidixic acid, tetracycline, enrofloxacin or streptomycin was less common (3-6%) and only occasional isolates were resistant to amoxicillin/clavulanic acid, gentamicin, neomycin or trimethoprim. No isolate was resistant to apramycin, ceftiofur or florfenicol.

Twenty-eight isolates (9%) were resistant to more than one antimicrobial with eleven of the tested substances represented in the patterns. Among all isolates of *E. coli* from years 2000, 2001 and 2002 the association between resistance

Table EC I. Occurrence of resistance (%) among isolates of *Escherichia coli* from chickens, 2002. Data for 2000 (chickens and cattle) and 2001 (chickens and pigs) are given for comparison (SVARM 2000 and 2001)

Substance	Range tested (mg/L)	Breakpoint resistance (mg/L)	Percent resistant 95% confidence interval inside brackets									
			Chickens						Pigs		Cattle	
			2002 n=306		2001 n=296		2000 n=274		2001 n=308		2000 n=293	
Amoxicillin/Clav[1]	2-16	>8	2	(0.5-3.8)	-[2]		-[2]		-[2]		-[2]	
Ampicillin	0.25-32	>8	4	(2.3-7.2)	3	(1.2-5.3)	5	(2.6-8.0)	3	(1.6-5.9)	0	(0.0-1.3)
Apramycin	0.25-32	>32	0	(0.0-1.2)	0	(0.0-1.2)	0	(0.0-1.3)	0	(0.0-1.2)	0	(0.0-1.3)
Ceftiofur	0.25-2	>2	0	(0.0-1.2)	0	(0.0-1.2)	0	(0.0-1.3)	0	(0.0-1.2)	0	(0.0-1.3)
Chloramphenicol	2-16	>16	0	(0.0-1.2)	0	(0.0-1.2)	<1	(0.1-2.6)	2	(0.5-3.8)	0	(0.0-1.3)
Enrofloxacin	0.03-4	>0.25	3	(1.6-5.9)	1	(0.2-2.9)	4	(1.8-6.6)	<1	(0.0-1.8)	0	(0.0-1.3)
Florfenicol	2-16	>16	0	(0.0-1.2)	0	(0.0-1.2)	0	(0.0-1.3)	0	(0.0-1.2)	0	(0.0-1.3)
Gentamicin	0.25-32	>8	<1	(0.0-1.8)	<1	(0.0-1.9)	<1	(0.0-2.0)	0	(0.0-1.2)	0	(0.0-1.3)
Nalidixic acid	1-128	>16	5	(2.5-7.6)	2	(0.6-3.9)	4	(2.3-7.5)	<1	(0.0-1.8)	<1	(0.1-2.4)
Neomycin	1-128	>8	2	(0.7-4.2)	<1	(0.0-1.9)	<1	(0.1-2.6)	0	(0.0-1.2)	0	(0.0-1.3)
Streptomycin	2-256	>32	4	(1.8-6.3)	2	(1.0-4.8)	4	(2.3-7.5)	9	(6.4-13.2)	5	(2.9-8.3)
Sulphametoxazole	64-512	>256	10	(6.7-13.7)	12	(8.4-16.1)	12	(8.1-16.0)	10	(6.7-13.6)	1	(0.4-3.5)
Tetracycline	0.5-64	>8	6	(3.3-8.8)	4	(2.4-7.4)	8	(4.8-11.5)	8	(5.6-12.1)	1	(0.4-3.5)
Trimethoprim	0.12-16	>8	<1	(0.0-1.8)	1	(0.2-2.9)	<1	(0.1-2.6)	2	(0.9-4.6)	0	(0.0-1.3)

[1] Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2/1 (amoxicillin/clavulanic acid). [2] Not given due to uncertainties in the analysis years 2000 and 2001.

to sulphonamides and tetracycline was statistically significant (P<0.05) (Table EC II). Resistance to sulphonamides in combination with resistance to ampicillin, nalidixic acid or streptomycin also occurred but the associations were not statistically significant (P>0.05).

Table EC II. Cross tabulation of succeptibility to sulphonamides and tetracycline in *Escherichia coli* isolated from chickens years 2000, 2001 and 2002 (n=876) (R=resistant; S=sensitive).

		Tetracycline	
		R	S
Sulphonamides	R	25	72
	S	26	753

Ten isolates (3%) were multiresistant, i.e. were resistant to three or more of the antimicrobials tested (Table EC III). All 25 multiresistant isolates from years 2000, 2001 and 2002 had the resistance traits sulphonamides, streptomycin, tetracyclines or nalidixic acid in their resistance patterns. Six isolates had all four traits included. In good agreement with the observed association between resistance to sulphonamides and tetracycline (see above), 16 of the multiresistant isolates were resistant to both these antimicrobials.

Overall, resistance levels were low in an international perspective and of the same magnitude as in years 2000 and 2001. Nor did the prevalence of isolates resistant to more than one antimicrobial differ from the prevalence in years 2000 and 2001. Sulphonamide resistance was the most prevalent trait, which could be a consequence of the occasional use of this substance to treat coccidiosis in broiler chicken production (SVARM 2000). By contrast, resistance to the other antimicrobials cannot be explained by direct selection as these substances are used in small amounts only (tetracyclines and fluoroquinolones) or not at all (aminoglycosides and ampicillin). However, the associations between sulphonamide resistance and other resistance traits, notably tetracycline, observed in the combined materials from 2000, 2001 and 2002 indicate that use of the former drug might co-select for resistance to the other substances.

Table EC III. Number of *Escherichia coli* resistant to three or more antimicrobials, presented by year and resistance phenotype, chickens 2002. "R" in hatched fields indicates resistance. Data for 2000 and 2001 are from SVARM 2000 and 2001

Year			Resistance pattern[1]										
2002 n=306	2001 n=296	2000 n=274	Su	Tc	Sm	Nal	Ef	Am	A/C[2]	Tr	Cm	Nm	Gm
		1	R	R	R	R	R				R		
1			R	R	R	R	R					R	
3		1	R	R	R	R						R	
		1	R	R	R			R		R		R	
1		1	R	R	R								
	1		R	R		R	R			R			
	1		R	R		R				R			
	1		R	R		R	R						
	1	1	R	R		R							
	1		R	R						R			
		1	R	R									R
	1		R		R	R						R	
		1	R		R			R			R		
1			R		R								R
		1		R	R	R	R						
1		1		R		R	R						
1				R				R	R				
1					R	R	R					R	
1					R	R	R						

Total

10 (3%)	6 (2%)	9 (3%)

[1] Su: sulphonamides; Tc: tetracycline; Sm: streptomycin; Nal: nalidixic acid; Ef: enrofloxacin; Am: ampicillin; A/C: amoxicillin/clavulanic acid Tr: trimethoprim; Cm: chloramphenicol; Nm: neomycin; Gm: gentamicin.

[2] Not included years 2000 and 2001 due to uncertainties in the analysis.

Table EC IV. Distribution of MICs for *Escherichia coli* from chickens year 2002 (n=306). Data for years 2000 (n=274) and 2001 (n=296) are given for comparison (SVARM 2000 and SVARM 2001). Bold vertical lines indicate breakpoint for resistance

Substance	Year	Percent resistant	Distribution (%) of MICs[1] (mg/L)															
			≤ 0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
Amoxicillin/ Clavulanic acid[2]	-02	2							16.3	66.3	15.7	0.7	1.0					
	-01	-[3]																
	-00	-[3]																
Ampicillin	-02	4					1.0	9.2	65.0	20.6		0.7		3.6				
	-01	3					0.3	5.7	47.6	43.2	0.3			2.7				
	-00	5						1.8	23.4	69.3	0.7			4.7				
Apramycin	-02	0							0.3	1.0	26.5	54.6	17.6					
	-01	0						0.3		0.7	29.7	59.1	10.1					
	-00	0							0.4	2.6	25.2	55.1	16.8					
Ceftiofur	-02	0				13.7	68.0	17.6	0.7									
	-01	0				16.9	72.0	11.1										
	-00	0				11.3	74.5	14.2										
Chloramphenicol	-02	0							1.6	70.3	27.8	0.3						
	-01	0							1.7	64.2	34.1							
	-00	<1								27.0	72.3		0.7					
Enrofloxacin	-02	3	20.9	68.6	6.2	1.0	2.6	0.7										
	-01	1	33.4	63.5	1.0	1.0	0.7			0.3								
	-00	4	19.3	75.2	1.1	0.7	2.2	1.5										
Florfenicol	-02	0							0.7	50.3	47.1	2.0						
	-01	0							1.4	49.0	49.0	0.7						
	-00	0								13.9	85.0	1.1						
Gentamicin	-02	<1					1.3	26.1	55.9	14.7	1.6		0.3					
	-01	<1					0.3	16.6	50.7	27.7	4.4	0.3						
	-00	<1						14.6	52.2	30.7	2.2	0.4						
Nalidixic acid	-02	5							20.3	69.6	5.6		0.3	1.0	1.3	2.0		
	-01	2							8.4	52.0	36.1	1.7			0.7	1.0		
	-00	4								23.7	66.8	5.1			1.1	3.3		
Neomycin	-02	2						1.3	36.3	52.0	8.5		0.3		1.3	0.3		
	-01	<1						1.4	51.7	40.9	5.7			0.3				
	-00	<1						1.1	51.5	40.5	6.2			0.7				
Streptomycin	-02	4								1.6	44.4	48.7	1.6	0.7	1.3	1.3	0.3	
	-01	2								2.0	56.4	35.8	3.4	1.0		0.7	0.7	
	-00	4								2.9	59.9	32.1	0.7	0.7	0.7	1.1	1.8	
Sulphamethoxazole	-02	10												64.7	25.5			9.8
	-01	12												64.2	23.0	1.0		11.8
	-00	12												32.5	54.4	1.5		11.7
Tetracycline	-02	6					1.0	31.0	49.7	11.8	1.0			0.3	5.2			
	-01	4						22.3	62.8	10.1	0.3	0.3			4.1			
	-00	8						5.1	59.9	27.0	0.4		0.4		7.3			
Trimethoprim	-02	<1			0.7	23.2	58.5	14.7	2.6				0.3					
	-01	1			1.7	20.6	59.5	15.9	1.0	0.3			1.0					
	-00	<1			2.6	8.0	55.5	32.1	1.1				0.7					

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration. [2] Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2/1 (amoxicillin/clavulanic acid). [3] Data not included due to uncertainties in the analysis years 2000 and 2001.

Enterococci

Results and comments

The material includes 332 isolates from broiler chickens. *Enterococcus faecium* (57%) was the predominant species followed by *E. faecalis* (17%) and *E. hirae* (14%) (Table ENT I). Other species of enterococci isolated were *E. mundtii* (2%) and *E. durans* (6%). About five percent of the isolates could not be typed to species level. Isolation frequencies were roughly equal to those for years 2000 and 2001.

All enterococci

Resistance to narasin was the most common resistance trait (72%) but levels of resistance to tetracycline, bacitracin or erythromycin were also high (20-27%) (Table ENT II). It should be observed that flavomycin and virginiamycin are not included in the overall comparison as the inherent susceptibility to these substances differs between species of enterococci.

No isolate obtained from direct culture was resistant to ampicillin. Further, no ampicillin resistant isolate was obtained in 212 samples collected in February to June after culture on selective media containing ampicillin (see Appendix 3 for details).

One isolate obtained from direct culture, an *E. faecium*, was resistant to vancomycin. However, all samples were also cultured in enrichment-broth containing vancomycin. From these cultures, vancomycin resistant enterococci (VRE) were isolated in 83 (24%) of 351 samples cultured. All isolates were *E. faecium* with MICs for vancomycin of >128 mg/L. In addition, all isolates were resistant to narasin and the majority had low-level resistance to erythromycin. Twenty-two isolates were tested by PCR and all carried the *vanA* gene-cluster. Similar antibiograms and patterns on subtyping using the PhenePlate" system indicate that the majority of isolates were closely related (see Appendix 3 for details).

Enterococcus faecalis

Most isolates of *E. faecalis* (80%) were resistant to at least one antimicrobial. Resistance to tetracycline was the most prevalent trait (58%) but resistance to bacitracin, erythromycin or narasin was also common (26-39%) (Table ENT V).

Thirty-one isolates (60%) were resistant to more than one antimicrobial. Seven antimicrobials were represented in the resistance patterns of these isolates, resistance against tetracycline, bacitracin, erythromycin or narasin were the most common traits. In the combined material from years 2000, 2001 and 2002 there were statistically significant associations between resistance to bacitracin and erythromycin (P<0.001), between bacitracin and tetracycline (P<0.001) and between narasin and erythromycin (P<0.001) (Table ENT III).

Ten isolates (18%) were multiresistant (Table ENT VII). Among the 41 multiresistant isolates in the combined material from years 2000, 2001 and 2002, 23 isolates (56%) were resistant to narasin, tetracycline and erythromycin. Of these isolates, 16 were resistant also to bacitracin.

Table ENT I. Prevalence of enterococci in samples of caecal content from chickens, 2002. Species not identified as *Enterococcus faecalis*, *E. faecium* or *E. hirae* are given as "other species". Data for years 2000 and 2001 are given for comparison (SVARM 2000 and SVARM 2001)

Year	Number of samples cultured	Percent positive cultures	Number of isolates tested for antimicrobial susceptibility	*Enterococcus* species isolated Number of isolates and percent of total isolates in brackets.			
				E. faecalis	*E. faecium*	*E. hirae*	Other species
2002	351	95%	332	57 (17%)	189 (57%)	45 (14%)	41 (12%)
2001	324	93%	302	49 16%)	204 (68%)	27 (9%)	22 (7%)
2000	317	82%	261	47 (18%)	151 (58%)	28 (11%)	35 (13%)

Table ENT II. Occurrence of resistance (%) among isolates of *Enterococcus* spp. chickens, 2002. Data for 2000 (calf and chicken) and 2001 (pig and chicken) are given for comparison (SVARM 2000 and SVARM 2001)

Substance	Range tested (mg/L)	Breakpoint resistance (mg/L)	Percent resistant 95% confidence interval inside brackets									
			Chickens						Pigs		Cattle	
			2002 n=332		2001 n=302		2000 n=261		2001 n=308		2000 n=277	
Ampicillin	0.25-32	>8	0	(0.0-1.1)	<1	(0.0-1.8)	0	(0.0-1.4)	<1	(0.1-2.6)	0	(0.0-1.3)
Avilamycin	0.5-32	>16	<1	(0.0-1.7)	0	(0.0-1.2)	0	(0.0-1.4)	0	(0.0-1.3)	<1	(0.0-2.0)
Bacitracin[1]	0.5-32	>32	22	(17.9-27.2)	16	(12.3-20.9)	20	(14.9-24.9)	1	(0.2-3.1)	<1	(0.1-2.6)
Erythromycin	0.25-32	>4	20	(16.3-25.5)	21	(16.1-25.5)	19	(14.6-24.5)	12	(8.0-15.8)	3	(1.0-5.1)
Flavomycin	2-128	>32	NR[2]		NR[2]		NR[2]		NR[2]		NR[2]	
Gentamicin	0.5-32, 512	>512	0	(0.0-1.1)	0	(0.0-1.2)	0	(0.0-1.4)	1	(0.2-3.1)	0	(0.0-1.3)
Narasin	0.12-16	>2	72	(66.8-76.8)	75	(69.6-79.6)	72	(65.8-77.0)	3	(1.3-5.6)	1	(0.4-3.7)
Neomycin	2-128, 1024	>1024	0	(0.0-1.1)	0	(0.0-1.2)	0	(0.0-1.4)	2	(0.6-4.1)	<1	(0.0-2.0)
Streptomycin	2-128, 1024	>1024	1	(0.3-3.1)	<1	(0.1-2.4)	2	(0.9-4.9)	7	(3.9-10.0)	<1	(0.1-2.6)
Tetracycline	0.25-32	>8	27	(22.4-32.2)	31	(25.6-36.3)	37	(30.9-43.0)	22	(17.5-27.6)	5	(3.1-8.8)
Vancomycin	1-128	>16	<1	(0.0-1.7)	0	(0.0-1.2)	0	(0.0-1.4)	0	(0.0-1.3)	0	(0.0-1.3)
Virginiamycin	0.5-64	>8	NR[2]		NR[2]		NR[2]		NR[2]		NR[2]	

[1] MIC in U/mL; [2] not relevant as susceptibility in some species of *Enterococcus* is inherently low.

Enterococcus faecium

The majority of the isolates (84%) were resistant to at least one antimicrobial. Resistance against narasin was the most prevalent trait (78%) (Table ENT V). Prevalence of resistance against tetracycline or bacitracin was 24-25% and against erythromycin or virginiamycin 11%.

Ninety-four isolates (50%) were resistant to more than one antimicrobial with seven substances represented in the patterns. Of these isolates, the vast majority (98%) were resistant to narasin. In the combined material from years 2000, 2001 and 2002 there were statistically significant associations between resistance against narasin and bacitracin ($P<0.01$) and between narasin and virginiamycin ($P<0.01$) (Table ENT IV). Further, resistance against tetracycline was associated with resistance to erythromycin ($P<0.05$) and to virginiamycin ($P<0.05$).

Twenty-three isolates (12%) of *E. faecium* were multiresistant (Table ENT VII). Of the 70 multiresistant isolates from years 2000, 2001 and 2002, narasin was included in the resistance phenotype of 68 isolates (98%). Further, among the 70 multiresistant isolates the combination narasin-tetracycline-bacitracin resistance was found in 27 (39%) and narasin-tetracycline-virginiamycin in 25 (36%). Seven isolates (10%) had all four traits (narasin-tetracycline-bacitracin-virginiamycin) in their phenotype.

Enterococcus hirae

The majority of isolates (93%) were resistant to at least one antimicrobial. Narsin resistance was the most prevalent trait (87%) followed by erythromycin resistance (40%) (Table ENT V). 21 isolates (47%) were resistant to more than one antimicrobial but only one isolate was multiresistant.

Comments in relation to previous years

Resistance of noticeable magnitude occurred to the same antimicrobials as in years 2000 and 2001. Overall, narasin resistance was the most prevalent trait. Around 80, 90 and 40 % of *E. faecium, E. hirae* and *E. faecalis,* respectively, were resistant to this antimicrobial. The high and stable levels of resistance to this antimicrobial agrees with the common use of narasin as coccidiostat in broiler production.

Tetracycline resistance was common in *E. faecalis* (58%) and in *E. faecium* (25%) although less prevalent than in years 2000 and 2001. The difference between years was statistically significant for *E. faecium* as evaluated by the Chi^2-test. Resistance to erythromycin was also of appreciable magnitude; in *E. faecalis* (26%), *E. faecium* (11%) and *E. hirae* (40%). In both *E. faecalis* and *E. faecium* levels of erythromycin resistance were lower year 2002 than in years 2000 and 2001 but the difference was not verified statistically. Bacitracin resistance was also common in both *E. faecalis* (35%) and *E. faecium* (24%). Resistance levels were higher 2002 than in the preceding two years but the differences were not statistically significant.

The high levels of resistance to tetracycline, erythromycin, bacitracin as well as occurrence of resistance to streptomycin in *E. faecalis* and to virginiamycin in *E. faecalis* and *E. hirae* cannot be explained by use of the respective substances. Macrolides and tetracyclines are seldom used in Swedish broiler production and virginiamycin and bacitracin have not been used since the 80s (SVARM 2000). Resistance to these antimicrobials can either be a remnant of the past use or the result of co-selection. In the combined data from years 2000, 2001 and 2002 there are indications of linked resistance genes in enterococci suggesting that use of one antimicrobial might select for resistance to others. Thus, some of the resistance traits observed, i.e. resistance to bacitracin or virginiamycin in *E. faecium* or resistance to erythromycin in *E. faecalis*, might be a consequence of the use of narasin. The closer associations between these resistance traits deserve further study.

Table ENT III. Cross tabulation of succeptibility to erythromycin versus narasin, erythromycin versus bacitracin and bacitracin versus tetracycline in *Enterococcus faecalis* isolated from broiler chickens years 2000, 2001 and 2002 (n=153). (R=resistant; S=sensitive)

		Narasin	
		R	S
Erythromycin	R	36	13
	S	28	76

		Bacitracin	
		R	S
Erythromycin	R	25	24
	S	21	83

		Bacitracin	
		R	S
Tetracycline	R	39	55
	S	46	52

Table ENT IV. Cross tabulation of succeptilbility to narasin versus bacitracin, narasin versus virginiamycin, tetracycline versus erythromycin and tetracycline versus virginiamycin in *Enterococcus faecium* isolated from broiler chickens year 2000, 2001 and 2002 (n=544). (R=resistant; S=sensitive)

		Narasin	
		R	S
Bacitracin	R	95	11
	S	335	103

		Narasin	
		R	S
Virginiamycin	R	51	4
	S	379	110

		Tetracycline	
		R	S
Erythromycin	R	29	40
	S	129	346

		Tetracycline	
		R	S
Virginiamycin	R	24	31
	S	134	386

The proportion of VRE among enterococci in the gastrointestinal tract of broiler chickens appears to be low in Sweden as in the years 2000-2002 only one isolate was obtained after direct culture of 992 samples. This concurs with the fact that avoparcin, an antimicrobial feed-additive selecting for vancomycin resistance, has not been used in Swedish animal production since the mid 1980s. In comparison, the prevalence in broiler chickens in Denmark and The Netherlands year 2001 were 3 and 5% respectively (DANMAP, 2001, Mevius & van Pelt, 2003). VRE are rare also in humans in Sweden and only 20 isolates were reported in 2001 (SWEDRES 2001).

The results of the selective cultures, with increased sensitivity to detect vancomycin resistant enterococci, however show that the resistance gene (*van*A) is present among enterococci in the gut of broiler chickens. In these cultures, VRE were isolated in 0.6, 7.4 and 23.6% of the samples in the years 2000, 2001 and 2002, respectively. Moreover, the prevalence of VRE after selective culture seems to have increased over the three years studied. This could partly be the result of improved skills in evaluation of the selective cultures but may also be a true increase in prevalence of flocks/production sites where VRE occur.

An explanation for the increased prevalence could be that VRE has been introduced into the flocks. In support of this is the fact that the majority of isolates from 2001 and 2002 had similar phenotypes both as antibiograms and by biochemical tests. Accordingly, the majority of isolates could belong to a single clone, which is in contrast to the situation in Norway and Denmark where several clones of VRE are isolated from broiler chickens (Borgen *et al*., 2000a, Heuer *et al*., 2002a). Hypothetically, a VRE-clone could have been introduced into buildings used for broiler production. VRE can persist in the environment for long periods of time without selection pressure exerted by avoparcin use. This was shown in Norway and Denmark where, after selective culture, VRE could be isolated in 99 and 74% of broiler flocks housed in buildings not exposed to avoparcin for several years (Borgen *et al*., 2000b, Heuer *et al.,* 2002b). Accordingly, it is probable that once introduced, VRE can be isolated from subsequent flocks of chickens housed in the same building.

To further investigate the epidemiology of VRE among Swedish broiler chickens, a study in collaboration with the Swedish Poultry Meat Association was initiated in the autumn of 2002. In the study, environmental samples from feed-mills and hatcheries and samples of pooled faeces from commercial broiler flocks and parent flocks are analysed by selective cultures. A positive culture indicates that VRE are present in the sample but does not provide information on the proportion of VRE of all enterococci in the sample nor on the prevalence among chickens in a flock. Preliminary data indicate that the prevalence of VRE on commercial broiler flock level is currently around 15%. VRE were not isolated in samples from the four feed-mills producing chicken feed in Sweden nor in samples from the four hatcheries or from the 28 flocks of parent birds that supplied eggs for hatching in the autumn of 2002. The results indicate that VRE are not continuously spread to chicken flocks through feed or by day old chickens. This however does not preclude that such a spread has occurred at a previous date. Further studies on the epidemiology of VRE among broiler chickens including means of containing its spread seems warranted.

Interestingly, only one of the 895 isolates of enterococci tested in years 2000, 2001 and 2002 was resistant to ampicillin. Furthermore, this resistance trait was not found despite culture on selective media of all samples collected in February to June year 2002. By contrast, in Sweden ampicillin resistant enterococci were found in about 20 and 6% of hospitalised and non-hospitalised patients, respectively (SWEDRES 2001). It seems unlikely that Swedish chickens are the source of these enterococci.

Table ENT V. Occurrence of resistance (%) among *Enterococcus faecalis, Enterococcus faecium* and *Enterococcus hirae* from chickens, presented by source of isolates and bacterial species, 2002. Data for 2000 (chickens and cattle) and 2001 (chickens and pigs) are presented for comparison (SVARM 2000 and SVARM 2001). Range of dilutions tested and breakpoints for resistance are given in Table ENT II

Substance	Chickens									Pigs			Cattle		
	E. faecalis			*E. faecium*			*E. hirae*			*E. faecalis*	*E. faecium*	*E. hirae*	*E. faecalis*	*E. faecium*	*E. hirae*
	2002 n=57	**2001** n=49	**2000** n=47	**2002** n=189	**2001** n=204	**2000** n=151	**2002** n=45	**2001** n=27	**2000** n=28	**2001** n=52	**2001** n=106	**2001** n=77	**2000** n=22	**2000** n=71	**2000** n=27
Ampicillin	0	0	0	0	<1	0	0	0	0	2	1	0	0	0	0
Avilamycin	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Bacitracin	35	31	23	24	15	20	2	4	7	0	3	0	0	1	0
Erythromycin	26	41	30	11	15	12	40	22	25	27	11	0	5	6	0
Flavomycin	2	6	11	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	2	NR[1]	NR[1]	14	NR[1]	NR[1]
Gentamicin	0	0	0	0	0	0	0	0	0	4	0	1	0	0	0
Narasin	39	45	43	78	80	79	87	89	89	4	4	3	0	1	2
Neomycin	0	0	0	0	0	0	0	0	0	6	2	0	0	0	0
Streptomycin	7	4	9	0	0	1	0	0	4	25	4	0	5	0	0
Tetracycline	58	67	60	25	27	38	7	4	7	63	7	10	14	6	<1
Vancomycin	0	0	0	<1	0	0	0	0	0	0	0	0	0	0	0
Virginiamycin	NR[1]	NR[1]	NR[1]	11	11	8	7	52	11	NR[1]	3	0	NR[1]	1	0

[1] Not relevant as susceptibility in some species of *Enterococcus* is inherently low.

Table ENT VI. Occurrence of resistance (%) among *Enterococcus faecalis, Enterococcus faecium* and *Enterococcus hirae* from chickens, presented by bacterial species and source of isolates, 2002. Data for 2000 (chickens and cattle) and 2001 (chickens and pigs) are presented for comparison (SVARM 2000 and SVARM 2001). Range of dilutions tested and breakpoints for recistance are given in Table ENT II

Substance	*E. faecalis*					*E. faecium*					*E. hirae*				
	Chickens			Pigs	Cattle	Chickens			Pigs	Cattle	Chickens			Pigs	Cattle
	2002 n=57	2001 n=49	2000 n=47	2001 n=52	2000 n=22	2002 n=189	2001 n=204	2000 n=151	2001 n=106	2000 n=71	2002 n=45	2001 n=27	2000 n=28	2001 n=77	2000 n=27
Ampicillin	0	0	0	2	0	0	<1	0	1	0	0	0	0	0	0
Avilamycin	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Bacitracin	35	31	23	0	0	24	15	20	3	1	2	4	7	0	0
Erythromycin	26	41	30	27	5	11	15	12	11	6	40	22	25	0	0
Flavomycin	2	6	11	2	14	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]
Gentamicin	0	0	0	4	0	0	0	0	0	0	0	0	0	1	0
Narasin	39	45	43	4	0	78	80	79	4	1	87	89	89	3	2
Neomycin	0	0	0	6	0	0	0	0	2	0	0	0	0	0	0
Streptomycin	7	4	9	25	5	0	0	1	4	0	0	0	4	0	0
Tetracycline	58	67	60	63	14	25	27	38	7	6	7	4	7	10	<1
Vancomycin	0	0	0	0	0	<1	0	0	0	0	0	0	0	0	0
Virginiamycin	NR[1]	NR[1]	NR[1]	NR[1]	NR[1]	11	11	8	3	1	7	52	11	0	0

[1] Not relevant as susceptibility in some species of *Enterococcus* is inherently low.

Table ENT VII. Number of isolates of *Enterococcus faecalis* (left panel) and Enterococcus faecium (right panel) resistant to three or more antimicrobials, presented by year and resistance phenotype, chickens 2002. "R" in hatched fields indicates resistance. Data for 2000 and 2001 from SVARM 2000 and 2001

E. faecalis								
Year			Resistance pattern[1]					
2002 n=57	2001 n=49	2000 n=47	Na	Tc	Em	Ba	Sm	Fl
3	7	2	R	R	R	R		
2		2	R	R	R	R	R	
		1	R	R	R		R	
1	3	1	R	R	R			
	1		R	R	R			R
	1	1	R	R				R
1	1		R	R		R		
1	1	1	R		R	R		
	3	1	R		R			R
1			R		R	R	R	
1			R			R	R	
		5		R	R	R		

Total		
10 (18%)	17 (35%)	14 (30%)

E. faecium									
Year			Resistance pattern[1]						
2002 n=189	2001 n=204	2000 n=151	Na	Tc	Em	Ba	Sm	Vi	Va
		1	R	R	R	R		R	
1		2	R	R	R	R			
1	1		R	R	R			R	
5	5	5	R	R	R				
2	2	2	R	R		R		R	
3	5	9	R	R		R			
3	7	5	R	R				R	
2	1		R		R	R			
1			R		R				R
2			R		R			R	
3			R			R		R	
		1		R	R	R			
		1			R		R	R	

Total		
23 (12%)	26 (14%)	21 (10%)

[1] Na: narasin; Tc: tetracycline; Em: erythromycin; Sm: streptomycin; Ba: bacitracin; Fl: flavomycin; Vi: virginiamycin; Va: vancomycin.

Table ENT VIII. Distribution of MICs for *Enterococcus faecalis* from chickens year 2002 (n=57). Data for years 2000 (n=47) and 2001 (n=49) are given for comparison (SVARM 2000 and SVARM 2001). Bold vertical lines indicate breakpoint for recistance

Substance	Year	Percent resistant	Distribution (%) of MICs[1] (mg/L)														
			≤ 0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Ampicillin	-02	0		1.8	3.5	89.5	5.3										
	-01	0		4.1	10.2	79.6	6.1										
	-00	0			8.5	70.2	19.1	2.1									
Avilamycin	-02	2				5.3	78.9	14.0			1.8						
	-01	0			2.0	2.0	57.1	36.7	2.0								
	-00	0			2.1		80.9	14.9	2.1								
Bacitracin[2]	-02	35				1.8		1.8	14.0	42.1	5.3	35.1					
	-01	31				2.0	2.0	8.2	26.5	16.3	14.3	30.6					
	-00	23				8.5	2.1	4.3	23.4	36.2	2.1	23.4					
Erythromycin	-02	26			24.6	7.0	36.8	5.3	8.8	1.8	1.8	14.0					
	-01	41		8.2	28.6	8.2	12.2	2.0	10.2	4.1		26.5					
	-00	30			10.6	21.3	27.7	10.6	4.3	4.3	4.3	17.0					
Flavomycin	-02	2					1.8	68.4	28.1					1.8			
	-01	6					2.0	67.3	20.4	4.1		2.0		4.1			
	-00	11					6.4	63.8	12.8	4.3	2.1		2.1	8.5			
Gentamicin	-02	0						3.5	31.6	50.9	12.3				1.8		
	-01	0				2.0	6.1	10.2	49.0	28.6	4.1						
	-00	0				2.1		6.4	36.2	44.7	10.6						
Narasin	-02	39	3.5	26.3	22.8		8.8	33.3	5.3								
	-01	45	12.2	18.4	10.2	4.1	10.2	24.5	14.3	6.1							
	-00	43	4.3	19.1	21.3	6.4	6.4	14.9	23.4	2.1	2.1						
Neomycin	-02	0								1.8	19.3	40.4	33.3			5.3	
	-01	0						4.1	2.0	18.4	32.7	16.3	18.4			8.2	
	-00	0							6.4	6.4	17.0	31.9	34.0			4.3	
Streptomycin	-02	7									3.5	45.6	40.4			3.5	7.0
	-01	4								4.1	24.5	53.1	12.2			2.0	4.1
	-00	9						2.1		2.1	8.5	51.1	27.7				8.5
Tetracycline	-02	58		1.8	1.8	24.6	8.8	1.8	3.5	19.3	29.8	8.8					
	-01	67			2.0	22.4	6.1	2.0		22.4	14.3	30.6					
	-00	60			2.1	25.5	12.8			12.8	21.3	25.5					
Vancomycin	-02	0				8.8	71.9	19.3									
	-01	0				14.3	73.5	12.2									
	-00	0				19.1	68.1	12.8									
Virginiamycin	-02	NR[3]				1.8	1.8	8.8	28.1	57.9	1.8						
	-01	NR[3]			2.0	2.0	8.2	14.3	22.4	42.9	8.2						
	-00	NR[3]			4.3	2.1	6.4	8.5	19.1	48.9	10.6						

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2]MIC in U/mL, see Appendix 3 for details; [3] Not relevant as susceptibility in *E. faecalis* is inherently low.

Table ENT IX. Distribution of MICs for *Enterococcus faecium* from chickens year 2002 (n=189). Data for years 2000 (n=151) and 2001 (n=204) are given for comparison (SVARM 2000 and SVARM 2001). Bold vertical lines indicate breakpoint for resistance

Substance	Year	Percent resistant	Distribution (%) of MICs[1] (mg/L)														
			≤ 0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Ampicillin	-02	0		4.8	21.7	29.1	27.5	12.7	4.2								
	-01	<1		15.7	19.6	24.0	25.0	10.8	4.4	0.5							
	-00	0		5.3	19.2	19.9	23.8	23.8	7.9								
Avilamycin	-02	0			1.6	10.6	52.4	30.7	4.8								
	-01	0			1.5	4.9	26.0	61.3	6.4								
	-00	0			0.7	4.0	32.5	57.0	6.0								
Bacitracin[2]	-02	24			1.6	19.6	3.7	4.8	11.1	21.2	13.8	24.3					
	-01	15			2.0	21.6	2.9	3.9	21.1	21.6	12.3	14.7					
	-00	20			2.0	10.6	4.6	4.0	25.8	22.5	10.6	19.9					
Erythromycin	-02	11		0.5	20.6	11.6	46.6	9.5	3.7	1.1	1.1	5.3					
	-01	15		9.3	17.2	33.3	16.7	8.8	2.9	0.5		11.3					
	-00	12		2.6	19.9	20.5	31.8	13.2	0.7	2.0		9.3					
Flavomycin	-02	NR[3]						1.6	2.6	3.7	3.2	3.7	4.8	80.4			
	-01	NR[3]						2.0	6.4	2.0	4.9	1.5	2.0	81.3			
	-00	NR[3]						0.7	4.0	3.3	2.6	1.3	2.0	86.1			
Gentamicin	-02	0				0.5	0.5	20.1	49.2	25.9	3.7						
	-01	0			0.5	1.0	6.4	22.1	51.5	17.6	1.0						
	-00	0					6.6	33.1	51.0	9.3							
Narasin	-02	78	0.5	2.6	3.7	6.3	9.0	36.5	39.7	1.6							
	-01	80	0.5	0.5	2.9	8.3	7.8	26.0	48.5	5.4							
	-00	79		0.7	6.6	3.3	9.9	23.2	53.6	2.6							
Neomycin	-02	0						10.6	34.4	36.0	15.9	2.6	0.5				
	-01	0					1.5	15.2	30.9	33.3	14.2	4.4	0.5				
	-00	0						9.3	45.7	27.2	11.3	4.6	2.0				
Streptomycin	-02	0						0.5		9.0	42.3	46.6	1.6				
	-01	0					0.5		0.5	15.2	47.1	34.3	2.5				
	-00	<1							0.7	14.6	59.6	21.9	2.6				0.7
Tetracycline	-02	25		1.6	17.5	52.4	1.6	1.6	0.5	5.8	7.4	11.6					
	-01	27		2.0	4.4	50.5	13.7	1.0	2.0	3.9	8.3	14.2					
	-00	38		0.7	6.0	47.0	6.0	1.3	1.3	8.6	10.6	18.5					
Vancomycin	-02	<1				76.2	18.5	4.2	0.5					0.5			
	-01	0				78.9	12.7	8.3									
	-00	0				79.5	14.6	4.6	1.3								
Virginiamycin	-02	11			18.5	27.5	29.1	0.5	13.2	10.6	0.5						
	-01	11			11.3	31.9	26.5	8.3	11.3	9.8	1.0						
	-00	8			11.3	29.1	31.1	4.0	16.6	6.6	1.3						

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] MIC in U/mL, see Appendix 3 for details; [3] Not relevant as susceptibility in *E. faecium* is inherently low.

Table ENT X. Distribution av MICs for *Entercoccus hirae* from chickens year 2002 (n=45). Data for years 2000 (n=28) and 2001 (n=27) are given for comparison (SVARM 2000 and SVARM 2001). Bold vertical lines indicate breakpoint for resistance

Substance	Year	Percent resistant	Distribution (%) of MICs[1] (mg/L)														
			≤ 0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Ampicillin	-02	0		51.1	33.3	8.9	6.7										
	-01	0		51.9	22.2	14.8	7.4		3.7								
	-00	0		28.6	10.7	17.9	39.3	3.6									
Avilamycin	-02	0				6.7	8.9	75.6	8.9								
	-01	0					14.8	70.4	11.1	3.7							
	-00	0				3.6	39.3	57.1									
Bacitracin[2]	-02	2				2.2	20.0	15.6	13.3	22.2	24.4	2.2					
	-01	4				11.1	18.5	11.1	11.1	7.4	37.0	3.7					
	-00	7				32.1	14.3	7.1	17.9	7.1	14.3	7.1					
Erythromycin	-02	40		8.9	48.9	2.2						40.0					
	-01	22		7.4	59.3	11.1			7.4			14.8					
	-00	25		21.4	35.7	7.1	3.6	7.1				25.0					
Flavomycin	-02	NR[3]					4.4	4.4	48.9	24.4	2.2			15.6			
	-01	NR[3]						11.1	55.6	7.4	3.7			22.2			
	-00	NR[3]						17.9	25.0		3.6	3.6		50.0			
Gentamicin	-02	0				2.2	33.3	46.7	6.7	6.7	4.4						
	-01	0					18.5	59.3	7.4	14.8							
	-00	0					25.0	17.9	25.0	21.4	10.7						
Narasin	-02	87		2.2	6.7		4.4	44.4	42.2								
	-01	89		3.7	7.4			18.5	63.0	7.4							
	-00	89			7.1	3.6		32.1	50.0	7.1							
Neomycin	-02	0					8.9	37.8	28.9	17.8	4.4		2.2				
	-01	0					3.7	29.6	33.3	18.5	7.4	3.7	3.7				
	-00	0					7.1	21.4	14.3	14.3	17.9	17.9	7.1				
Streptomycin	-02	0						2.2		13.3	68.9	8.9	6.7				
	-01	0								3.7	66.7	18.5	11.1				
	-00	4								17.9	35.7	21.4	21.4				3.6
Tetracycline	-02	7		2.2	4.4	73.3	13.3			2.2	2.2	2.2					
	-01	4			3.7	25.9	66.7					3.7					
	-00	7			7.1	82.1	3.6			3.6		3.6					
Vancomycin	-02	0				95.6	4.4										
	-01	0				88.9	11.1										
	-00	0				89.3	10.7										
Virginiamycin	-02	7					64.4	2.2	26.7	6.7							
	-01	52				3.7	37.0		7.4	33.3	18.5						
	-00	11				7.1	60.7	3.6	17.9	10.7							

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] MIC in U/mL, see Appendix 3 for details; [3] Not relevant as susceptibility in *E. hirae* is inherently low.

Data emanate, if not otherwise stated, from antimicrobial susceptibility testing of isolates from routine bacteriological examination at SVA of clinical submissions or post-mortem samples. Standard diagnostic methods were used for isolation and identification, and isolates were tested for antimicrobial susceptibility by a microdilution method (VetMIC™). Various panels of VetMIC™ with different antimicrobials and dilutions were used depending on bacterial species. For further details, see Appendix 3.

It is to be observed that some breakpoints for resistance used in previous SVARM-reports have been changed. To facilitate comparisons when data from previous years are presented, levels of resistance have been recalculated using the current breakpoints. For a summary of breakpoints used see Appendix 3.

Pig

Isolates included

Escherichia coli for the years 1992-2002 were isolated from clinical submissions of gastro-intestinal tract samples (gut content, faecal samples or mesenteric lymph nodes), while data from years 1989-91 includes all *E. coli* isolated from pigs, irrespective of type of material cultured. *Brachyspira hyodysenteriae* isolates emanate from clinical submissions of faecal samples. All isolates of *B. hyodysenteriae* obtained in pure culture were tested for susceptibility using a specially adapted broth dilution method (see Appendix 3 for details).

Isolates from all parts of Sweden are included. No information on the herds from which the isolates originate was available but most isolates of *E. coli* and *B. hyodysenteriae* are probably from herds with diarrhoeal problems. This implies that the data presented might not be representative of bacterial populations in general. However, these biases are probably inherent throughout the period and assessment of trends for *E. coli*, for which the material is large enough, appears relevant.

Results and comments

Escherichia coli

Resistance to streptomycin, tetracycline, trimethoprim-sulphonamide or ampicillin were the most common traits throughout the observation period (Table Pig I). All these substances are available as therapeutics in pig production but tetracyclines and trimethoprim-sulphonamide are probably the most commonly used. Levels of tetracycline resistance have been stable over the period studied and streptomycin resistance has decreased. In contrast, an increasing trend in resistance to ampicillin and to trimethoprim-sulphonamide was noted from 1998 and onwards. It is uncertain whether the changes in occurrence of resistance reflect a change in use of these substances. The total sales of aminoglycosides authorised for veterinary use have decreased steadily over the last 20 years (SVARM 2001). In contrast, sales of aminopenicillins have increased. Most of the sales of aminopenicillins is in the form of tablets, and is believed to be sold mainly for use in pets. However, in 1998 an injectable product containing amoxicillin, authorised for pigs and dogs, was launched on the Swedish market. Possibly, this product has, at least to some extent, replaced penicillin for treatment of arthritis in pigs.

Resistance occurred to the same antimicrobials as in isolates from healthy pigs sampled at slaughter, but at considerably higher levels (see Resistance in indicator bacteria). Moreover, resistance to more than one antimicrobial was more common in isolates from diagnostic submissions (31%) than in isolates from healthy animals (10%) (SVARM 2001). Thus, co-selection is likely to be of greater importance for the overall prevalence of resistance in isolates from sick than from healthy animals. Notably, in the periods 1992-95, 1996-99 and 2000-02 the prevalence of isolates resistant to both trimethoprim-sulphonamide and ampicillin was 4, 5 and 10%, respectively. Possibly, the increase in resistance to these antimicrobials could be the results of co-selection by use of either trimethoprim-sulphonamide or ampicillin.

Higher levels of resistance among isolates from diagnostic submissions than in isolates from healthy pigs probably reflect that the former isolates emanate from herds with diarrhoeal problems where antimicrobials are used to treat the infections. The material from diagnostic submissions is to a considerable extent composed of virulent strains of *E.coli*. These strains are likely to spread both within herd and between herds, and thereby resistance resulting from use of antimicrobials in one herd may spread to other herds. Thus, higher levels of resistance than in isolates originating from pigs sampled at slaughter are expected. Moreover, the material from diagnostic submissions is mostly composed of samples from pigs less than four months old, whereas pigs sampled at slaughter are approximately six months old. The presented data must be viewed in the light of these differences in sampled populations.

Table Pig I. Occurrence of resistance among *Escherichia coli* in pigs the years 1989-91, 1992-93, 1994-95, 1996-97, 1998-99, 2000-01 and 2002 and distribution of MICs among the isolates from 2002. All isolates are from the gastro-intestinal tract, isolated in samples for diagnostic submissions or from post mortem investigations. Bold vertical lines indicates breakpoint for resistance

Substance	Percent resistant							Distribution (%) of MICs[1] 2002 (mg/L)									
	1989-91 n=248	1992-93 n=295	1994-95 n=433	1996-97 n=958	1998-99 n=740	2000-01 n=666	2002 n=340	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	7	8	10	10	9[8]	15	19				16.2	52.4	10.6	1.8	19.1		
Ceftiofur	-	-	-	-	-	0[9]	0		37.6	52.6	9.4	0.3					
Chloramphenic.	2	4	7	6[6]	8	10[10]	-										
Enrofloxacin	1[3]	5	8[5]	4	5	6[11]	7	90.3	2.4	3.2	1.2	2.9					
Florfenicol	-	-	-	-	-	0[9]	<1[14]					2.9	44.0	49.0	3.5	0.6	
Gentamicin	1	<1[4]	<1	<1[6]	<1	<1[12]	1					80.6	14.4	3.8	0.9	0.3	
Neomycin	6	4	10	6	6	4[13]	4[15]						85.5	10.4	0.6		3.6
Nitrofurantoin	4	4	5	4[6]	3	4[10]	-										
Streptomycin	44	43	39	31	31	29	33						11.5	27.9	18.8	9.1	32.6
Tetracycline	28	31[4]	34	32	33[8]	34[11]	28				28.8	28.8	10.0	4.4	27.9		
Trim-Sulph[2]	17	15	15	13[7]	14[8]	16	21			75.0	3.2	0.3	0.3	21.2			

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); [3] 227 isolates tested; [4] 294 isolates tested; [5] 431 isolates tested; [6] 957 isolates tested; [7] 955 isolates tested; [8] 739 isolates tested, [9] 80 isolates tested, [10] 585 isolates tested, [11] 685 isolates tested, [12] 663 isolates tested, [13] 661 isolates tested, [14] 339 isolates tested, [15] 337 isolates tested.

Brachyspira hyodysenteriae

The breakpoints for antimicrobial resistance for *B. hyodysenteriae*, tentatively denoted in Table Pig II, are based on the MIC distribution for the tested isolates. The level of resistance to tylosin was high (73%) and of similar magnitude as in last year's survey. Notably, resistance to tylosin appears to have increased substantially since 1988-90 when 20 % of the isolates, tested with an agar dilution technique, had MICs >16 mg/L (Gunnarsson *et al.*, 1991). Tylosin resistance in *B. hyodysenteriae* is caused by a single point mutation in the 23S rRNA gene. This mutation also causes lincosamide resistance (Karlsson *et al.*, 1999).

No resistance to tiamulin was observed in the isolates from year 2002. Recent reports from Germany and the Czech republic show a progressively decreased susceptibility to tiamulin among *B. hyodysenteriae* isolates (Karlsson *et al.*, 2002; Cizek *et al.*, 2002). This emphasises that special attention should be paid to emergence of isolates with decreased susceptibility to tiamulin, especially as the therapeutic arsenal available to treat infections with *B. hyodysenteriae* is limited to few antimicrobials. This aspect is accentuated considering the increase in use of the pleuromutilins between 2001-2002 (see Use of antimicrobials).

Table Pig II. Occurrence of resistance among *Brachyspira hyodysenteriae* in pigs years 2000, 2001 and 2002 and distribution of MICs among the isolates from 2002. Isolates emanate from diagnostic submissions of faecal samples. Bold vertical lines indicate breakpoint for resistance

Substance	Breakpoint resistance (mg/L)	Percent resistant			Distribution (%) of MICs[1] 2002 (mg/L)													
		2000 n=50	2001 n=75	2002 n=109	≤0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tiamulin	>2	0	0	0		17.4	21.1	38.5	10.1	8.3	4.6								
Tylosin	>16	72	83	73								1.8	18.3	7.3		0.9			71.6

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration.

Cattle

Isolates included

Escherichia coli for the years 1992-2002 were isolated from clinical submissions of gastro-intestinal tract samples (gut content, faecal samples or mesenteric lymph nodes). No information on the age of the sampled animals was available, but it is probable that the majority of the sampled animals were less than one year old.

Udder pathogens (*Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae*) were isolated from milk samples from dairy cows with acute mastitis. Presented data are preliminary results from a project where milk samples from cases of acute mastitis were collected and cultured by practicing veterinarians. For further details on methodology see Appendix 3.

Results and comments

Escherichia coli

In *E. coli* from the gastro-intestinal tract of cattle, the most prevalent resistance traits were streptomycin (42%), tetracycline (31%), ampicillin (24%), the combination trimethoprim-sulphonamide (11%), enrofloxacin (10%) and chloramphenicol (9%) (Table Cattle I). No trends over time were apparent when levels of resistance were calculated for individual years (data not shown). Notably, levels of resistance were much higher than in *E. coli* isolated from healthy animals year 2000 (see Resistance in indicator bacteria).

Overall 46% of the isolates were susceptible to all antimicrobials tested. Resistance to two substances was observed in 13% of the isolates and 21% were multiresistant i.e. resistant to three or more substances. Of the multiresistant isolates, the majority, 96%, were resistant to both streptomycin and tetracycline in combination with other antimicrobials and 79% were resistant to streptomycin, tetracycline and ampicillin. Moreover, the 18 isolates resistant to chloramphenicol were all resistant to streptomycin and 16 also to tetracycline. In isolates from healthy cattle, occurrence of resistance to more than one substance was rare (<2%) (SVARM 2000).

The striking difference in levels of resistance between isolates from healthy animals and isolates from diagnostic submissions is probably because a large proportion of the latter most likely emanate from herds with disease problems. In such herds a high selection pressure, exerted by use of antimicrobial drugs, can be anticipated.

The antimicrobials against which appreciable levels of resistance were observed are to some extent used for therapy of cattle. The exception is chloramphenicol, which is not used, and where resistance probably results from co-selection by other substances. Moreover, as multiresistance is common it is likely that co-selection is an important factor in selection for resistance also to the other antimicrobials. Oral treatment of enteritis with aminoglycosides is probably a common practice in Sweden. As streptomycin resistance occurred in the majority of isolates resistant to more than one antimicrobial, this practice might be important for the overall levels of resistance observed.

Table Cattle I. Occurence of resistance and distribution om MICs among *Escherichia coli* from cattle the years 1992-02. All isolates are from the gastro-intestinal tract, isolated in samples for diagnostic submissions or from post mortem investigations. Bold vertical lines indicate breakpoint for resistance

Substance	Percent resistant	Distribution (%) of MICs[1] 1992-02 (mg/L)								
	1992-02 n=220	≤0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	24				23.2	36.4	16.4	24.1		
Ceftiofur	0[3]	31.3	56.3	12.5						
Chloramphenicol	9[4]				2.9	31.9	55.4	1.0	8.8	
Enrofloxacin	10	90.0	4.1	5.0	0.9					
Florfenicol	0[3]				6.3	56.3	37.5			
Gentamicin	1				40.5	54.5	4.1	0.5	0.5	
Neomycin	8					50.9	41.4	1.4		6.4
Nitrofurantoin	<1[4]							93.1	6.4	0.5
Streptomycin	42					1.0	33.2	19.5	4.1	42.3
Tetracycline	31			4.5	1.8	55.5	6.8	31.4		
Trim-Sulph[2]	11		87.3	1.8	0.5		10.5			

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); [3] 16 isolates tested; [4] 204 isolates tested.

Udder pathogens

Among *Staphylococcus aureus*, 7% of the isolates were resistant to penicillin due to ß-lactamase production. Notably, one of these isolates was resistant also to clindamycin, erythromycin, spiramycin, chloramphenicol and streptomycin. All isolates of *Streptococcus dysgalactaie* were sensitive to penicillin with MICs ≤0.06 mg/L. Resistance in this species was observed only against tetracycline (6%). Likewise, all *Streptococcus uberis* were sensitive to penicillin but 2% were resistant to tetracycline.

Overall, levels of resistance were low and in agreement with those reported for mastitis pathogens isolated from cases of acute mastitis in Sweden years 1994-95 (Nilsson *et al.*, 1997). Thus, no trends in resistance since the middle of the 90's among gram-positive mastitis pathogens can be discerned. For instance, the prevalence of ß-lactamase producing *S. aureus* (7%) was very similar to the level (6%) reported in the study from 1994-95.

The prevalence of ß-lactamase producing *S. aureus* was however higher, 18%, in the material presented in SVARM 2001. In contrast to the material presented here, last year's material included isolates from cases of subclinical and chronic mastitis. It is likely that ß-lactamase producing *S. aureus* are more prevalent in these types of mastitis than in acute infections.

Occurrence of ß-lactamase producing *S. aureus* resistant also to macrolides-lincosamides is worrying. *S. aureus* with this resistance phenotype has previously not been isolated from cases of mastitis in Sweden and are apparently rare. The finding emphasises the need for bacteriological diagnosis and subsequent susceptibility testing of isolates in mastitis therapy and prophylaxis. Thereby occurrence of such clones in dairy herds can be detected and measures to counteract its spread, within and between herds, can be taken.

Table Cattle II. Occurrence of resistance and distribution of MICs among *Staphylococcus aureus* isolated from acute, clinical mastitis in dairy cows year 2002-03. Bold vertical lines indicate breakpoint for resistance

Substance	Percent resistant n=100	Distribution (%) of MICs[1] (mg/L)													
		≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Avilamycin	0				1.0		4.0	65.0	29.0	1.0					
Cephalothin	0		27.0	66.0	7.0										
Chloramphenicol	1						1.0	11.0	87.0		1.0				
Clindamycin	1					98.0	1.0			1.0					
Erythromycin	1			2.0	55.0	41.0	1.0	1.0							
Gentamicin	0			11.0	52.0	30.0	7.0								
Neomycin	0					89.0	7.0	3.0	1.0						
Oxacillin[3]	1				35.0	52.0	12.0		1.0						
Penicillin	7[2]	76.0	17.0		1.0			1.0		5.0					
Spiramycin	1							5.0	5.0	70.0	19.0	1.0			
Streptomycin	1						8.0	41.0	41.0	7.0	2.0		1.0		
Tetracycline	0				95.0	5.0									
Trim-Sulpha[4]	0			97.0	3.0										
Vancomycin	0					96.0	4.0								
Virginiamycin	0				67.0	31.0	2.0								

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] denotes ß"-lactamase production [3] tested with 2% NaCl; [4] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole).

Table Cattle III. Occurrence of resistance and distribution of MICs among *Streptococcus dysgalactiae* isolated from acute, clinical mastitis in dairy cows year 2002-03. Bold vertical lines indicate breakpoint for resistance

Substance	Percent resistant n=100	Distribution (%) of MICs[1] (mg/L)													
		≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Avilamycin	0				44.0	32.0	20.0	1.0	1.0	2.0					
Cephalothin	0		71.0	28.0	1.0										
Chloramphenicol	0						31.0	66.0	3.0						
Clindamycin	0					100.0									
Erythromycin	0			97.0	3.0										
Gentamicin	NR[3]						7.0	32.0	43.0	17.0	1.0				
Neomycin	NR							4.0	17.0	44.0	32.0	2.0	1.0		
Penicillin	0	100.0													
Spiramycin	0							99.0	1.0						
Streptomycin	NR							2.0	18.0	55.0	21.0	4.0			
Tetracycline	6				4.0	28.0	44.0	13.0	5.0	2.0	1.0	3.0			
Trim-Sulpha[2]	0			95.0	5.0										
Vancomycin	0					100.0									
Virginiamycin	0				99.0	1.0									

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); [3] Not relevant as the inherent susceptibility is such that the MIC range is above concentrations that can be obtained during therapy.

Table Cattle IV. Occurrence of resistance and distribution of MICs among *Streptococcus uberis* isolated from acute, clinical mastitis in dairy cows years 2002-03. Bold vertical lines indicate breakpoint for resistance

Substance	Percent resistant n=98	Distribution (%) of MICs[1] (mg/L)													
		≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Avilamycin	0				4.1	23.5	58.2	14.3							
Cephalothin	0		57.1	39.8	2.0	1.0									
Chloramphenicol	0						15.3	82.7	2.0						
Clindamycin	0					100.0									
Erythromycin	0			100.0											
Gentamicin	NR[3]			1.0					4.1	17.3	63.3	14.3			
Neomycin	NR					1.0			1.0	2.0	7.1	51.0	37.8		
Penicillin	0	93.9	4.1	2.0											
Spiramycin	0							99.0		1.0					
Streptomycin	NR[4]								1.0		3.1	31.3	61.5	3.1	
Tetracycline	2				96.9	1.0						2.0			
Trim-Sulpha[2]	0			93.9	6.1										
Vancomycin	0					100.0									
Virginiamycin	0				99.0	1.0									

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/

Horse

Isolates included

Streptococcus zooepidemicus were isolated from bacteriological samples from the respiratory tract and *Escherichia coli* from samples from the female genital tract.
All isolates originate from diagnostic submissions and exclusion of repeated isolated from the same individual or stable was not possible. The data set is likely to represent the central-east part of Sweden rather than the whole country. Further, the data are probably biased towards treatment failures and recurrent infections. However, as these biases are assumed to be of similar magnitude throughout the period studied, assessment of trends of resistance frequencies appears relevant.

Results and comments

Streptococcus zooepidemicus
Among *S. zooepidemicus*, resistance to the combination trimethoprim-sulphonamide was the most common trait (28%) (Table Horse I). The level of resistance to this antimicrobial combination has increased markedly since the beginning of the 90s, probably a consequence of an increased therapeutic use of trimethoprim-sulphonamide formulations for oral use. In year 2002, however, the level of resistance was lower than in the last four years. Whether this represents a true decrease or is an effect of differences in the populations of horses sampled cannot be determined. The number of *S. zooepidemicus* isolated at SVA, as well as the proportion of these isolates tested for antimicrobial susceptibility (about 40%), are of similar magnitude years 2001 and 2002.Thus, there was no obvious bias in selection of isolates for testing between these two years. Resistance to other antimicrobials was rare year 2002 as well as over the whole period studied. Resistance to tetracycline however was more frequent (8%) year 2002 than in previous years. Whether this reflects a true increase cannot be determined. *S. zooepidemicus* has an inherent low susceptibility to aminoglycosides (gentamicin, neomycin) and therefore, assessment of resistance levels is not relevant for these substances.

Escherichia coli
In *E. coli,* the levels of resistance year 2002 were of similar magnitude as in years 1997-2000 and 2001(Table Horse II). The predominant resistance traits were trimethoprim-sulphonamide and streptomycin, 19 and 16%, respectively. Resistance to ampicillin or tetracycline occurred in 10 and 7% of the isolates, respectively.
Frequencies of resistance to ampicillin or streptomycin are lower from 1997 and onwards than in the first part of the 90s. For ampicillin, the level of resistance has stabilised around 10% whereas the level of streptomycin resistance year 2002 is the lowest during the whole observation period. In contrast, resistance to trimethoprim-sulphonamide has increased gradually but this does not parallel the striking increase in resistance to this drug combination observed among *S. zooepidemicus* in the same period of time. Of the total number of isolates from years 1992-2002, 69% were susceptible to all antimicrobials tested. Resistance to two substances occurred in 10% of the isolates and 9% were multiresistant, i.e. resistant to three or more substances. A high proportion of these multiresistant isolates, 72%, were resistant to trimethoprim-sulphonamide, streptomycin and ampicillin.
Interestingly, tetracycline resistance was less common in isolates of *E. coli* from horses than from pigs, cattle or dogs. Conversely, resistance to gentamicin, albeit low, was more common among isolates from horses and dogs than from the other animal species. This is consistent with the limited use of tetracyclines in horses and the fact that gentamicin is authorised for, and used in, horses but is not available for use in pigs and cattle in Sweden.

Table Horse I. Occurrence of resistance among *Streptococcus zooepidemicus* from horses the years 1992-96, 1997, 1998-99, 2000, 2001 and 2002 and distribution of MICs among the isolates from 2002. All isolates are from diagnostic submissions of samples from the respiratory tract. Bold vertical lines indicate breakpoint for resistance

Substance	Percent resistant						Distribution (%) of MICs[1] 2002 (mg/L)									
	1992-96 n=496	1997 n=125	1998-99 n=120	2000 n=301	2001 n=188	2002 n=163	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	<1	0	0	0	0	0				98.2	1.2	0.6				
Ceftiofur	-	-	-	-	-	0		98.2	1.2		0.6					
Chloramphenicol	<1	2	0	<1	<1[7]	-										
Clindamycin	<1	<1	0	<1[5]	<1[7]	-										
Erythromycin	1	2	<1	<1	<1[7]	-										
Florfenicol	-	-		-	-	1					80.4	13.5	3.7	1.2	1.2	
Gentamicin	NR[3]	NR	NR	NR	NR	NR					1.2		1.2	28.8	68.7	
Neomycin	NR	NR	NR	NR	NR	NR						1.2	0.6	1.2	22.1	74.8
Penicillin	<1	0	0	0	0	0	97.5		1.8	0.6						
Spiramycin	<1	<1	<1[4]	<1	<1	2						94.5	2.5	1.2		1.8
Tetracycline	3	4	3	4	3	8				44.2	38.0	7.4	2.5	8.0		
Trim-Sulph[2]	3	22	52	58[6]	43	28			57.7	9.2	3.7	1.8	27.6			

[1] Hatched fields denote range of dilutions tested for each substance; MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); [3] Not relevant as the inherent susceptibility is such that the MIC range is above concentrations that can be obtained during therapy; [4] 117 isolates tested; [5] 300 isolates tested; [6] 299 isolates tested; [7] 147 isolates tested.

Table Horse II. Occurrence of resistance among *Escherichia coli* from horses the years 1992-96, 1997-2000, 2001 and 2002 and distribution of MICs among the isolates from 2002. All isolates are from diagnostic submissions of samples from the female genital tract. Bold vertical lines indicate breakpoint for resistance

Substance	Percent resistant				Distribution (%) of MICs[1] 2002 (mg/L)									
	1992-96 n=176	1997-00 n=323	2001 n=103	2002 n=166	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	19	11	10	10				5.4	63.3	19.3	1.8	10.2		
Ceftiofur	-	-	-	0		24.1	66.9	7.8	1.2					
Chloramphenicol	2	2	3	-										
Enrofloxacin	5	5	5[4]	1	96.4	2.4	1.2							
Florfenicol	-	-	-	<1					4.2	31.9	60.8	2.4	0.6	
Gentamicin	3	4	2	4					67.5	25.3	3.6		3.6	
Neomycin	6	6	3	4						89.8	6.0	1.2		3.0
Nitrofurantoin	2	2	2	-										
Streptomycin	31	20	20	16						6.6	35.5	37.3	4.2	16.3
Tetracycline	7	7	8	7				40.4	39.2	10.2	3.0	7.2		
Trim-Sulph[2]	12[3]	16	18	19			74.1	3.6	1.2	2.4	18.7			

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); [3] 175 isolates tested; [4] 102 isolates tested.

Dog

Isolates included

Results of antimicrobial susceptibility tests of *Staphylococcus intermedius*, isolated from samples from skin, and of *Escherichia coli* isolated from urine are presented.

All isolates emanate from diagnostic submissions and might include repeat isolates from the same patients. Probably isolates from dogs in the central-eastern part of Sweden are over-represented. Further, it is likely that there is a bias towards isolates from dogs with recurrent disease or from therapeutic failures. Nonetheless, assuming that these biases are inherent throughout the period studied, inferences regarding trends seem relevant.

Results and comments

Staphylococcus intermedius

In isolates of *S. intermedius*, levels of resistance to penicillin (ß-lactamase production) have remained high (70-80%) over the period studied and similar rates were reported already in 1978 (Table Dog I). Thus, penicillinase sensitive penicillins are not likely to be efficient for treatment of recurrent pyodermas in dogs. However, that group of antimicrobials is widely used for other indications, which may explain the stable maintenance of this resistance determinant in canine staphylococci. Resistance in occasional isolates to cephalotin or oxacillin is probably due to methodological errors or to high levels of ß-lactamase production, and not to acquisition of the *mec*A-gene. Resistance to macrolides (erythromycin and spiramycin), lincosamides (clindamycin) or tetracycline is common (18–30%) and seems to have increased over the monitored period until year 2000 when the highest levels of resistance to these antimicrobials were observed. However, levels of resistance to erythromycin, but not clindamycin, are lower in year 2002 than in the preceding two years. Tetracycline resistance has also decreased since year 2000. By contrast, the level of resistance to the combination trimethoprim-sulphonamide is higher year 2002 (10%) than in previous years (1-3%). Interpretation of these changes in occurrence of resistance should be made with caution and trends must be verified in future materials.

High levels of resistance to macrolides and lincosamides concur with earlier reported findings (Sternberg, 1999, Holm *et al.* 2002). These antimicrobials are commonly prescribed to dogs (Odensvik *et al.*, 2001) and it is plausible that occurrence of resistance is related to this use. In staphylococci, *erm* genes commonly convey resistance to macrolides. Inducible expression of *erm*-genes conveys resistance to erythromycin only, while constitutive expression also leads to resistance to lincosamides. The above noted decrease in resistance to erythromycin with unchanged levels of clindamycin resistance could mean that inducibly expressed macrolide resistance has decreased, possibly following decreased use of erythromycin in dogs, while constitutively expressed resistance is maintained by use of lincosamides. In year 2002, most of the isolates that were macrolide resistant were also resistant to lincosamides (85%), compared with 52-72% in previous years.

Of the total number of isolates resistant to macrolides-lincosamides, 52% were also resistant to tetracycline. Isolates with this resistance phenotype comprise 5% of the total material. An association between resistance to these antimicrobials in *S. intermedius* from dogs is consistent with earlier observations from similar materials (Hansson *et al.*, 1997) and from a prospective study of isolates from pyoderma (Holm *et al.* 2002).

Table Dog I. Occurrence of resistance among *Staphylococcus intermedius* in dogs the years 1992-93, 1995, 2000, 2001 and 2002 and distribution of MICs for the isolates from 2002. All isolates are from diagnostic submissions of samples from skin. Bold vertical lines indicate breakpoint for resistance

Substance	Percent resistant					Distribution (%) of MICs[1] 2002 (mg/L)									
	1992-93 n=204	1995 n=94	2000 n=145	2001 n=156	2002 n=133	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Cephalothin	<1[4]	0[4]	<1[4]	0[4]	2					97.7	0.8		0.8	0.8	
Chloramphenicol	2	1	3	4	-										
Clindamycin	12	13	22	18	17				78.9		3.8	17.3			
Enrofloxacin	-	-	-	4[7]	2	64.7	29.3	4.5	0.8	0.8					
Erythromycin	19	25	30	28	20			77.4	2.3			20.3			
Gentamicin	<1	1	1[6]	0	0					100.0					
Neomycin	<1	1	0	0[8]	-										
Nitrofurantoin	1	0	<1	<1	<1								98.5	0.8	0.8
Oxacillin	1	0	1	<1	2			97.7		2.3					
Penicillin[2]	80[5]	72	79	82	78										
Spiramycin	20	25	30	26[8]	-										
Tetracycline	24	25	33	25[9]	25				72.9	2.3			24.8		
Trim/Sulph[3]	1	2	2	3	10			61.7	24.8	3.8	0.8	9.0			

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] denotes ß-lactamase production and/or MIC >0.12 mg/L; [3] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); [4] Breakpoint for resistance >4 mg/L; [5] 200 isolates tested; [6] 144 isolates tested; [7] 47 isolates tested; [8] 114 isolates tested; [9] 155 isolates tested.

Escherichia coli

Levels of resistance among *E. coli* were mostly of similar magnitude year 2002 as in previous years (Table Dog II). Resistance against ampicillin, streptomycin, tetracycline or the combination trimethoprim-sulphamethoxazole are the most common traits occurring in 10-20 % of the isolates. The frequency of resistance to tetracycline was numerically lower in 2000-2002 compared to previous years, which may represent a downward trend. With the possible exception of streptomycin, all these antimicrobials are commonly used for pets. The figures presented are of similar magnitude as those in a study from 1993 (Franklin *et al.*, 1993).

Levels of resistance to fluoroquinolones (enrofloxacin) are high throughout the observed period (8-12%). However, it must be observed that the breakpoints chosen for this report are based on microbiological criteria. Thus, if the breakpoint recommended by, eg, NCCLS is used, levels of resistance will be lower. Nonetheless, isolates where MICs of enrofloxacin exceed 0.25 mg/L are distinctly different from the wild type (susceptible) part of the population and can be assumed to have acquired resistance through mutations or other mechanisms. In 1998, around 24 000 prescriptions of fluoroquinolones for dogs were dispensed in Swedish pharmacies (Odensvik *et al.*, 2001). This corresponds to about 31 prescriptions of fluoroquinolones per 1000 dogs, and this comparatively high incidence of use probably explains the high levels of resistance in *E. coli* from dogs. Several new fluoroquinolones have recently been introduced on the Swedish market, and both the use and resistance levels need to be monitored closely.

The inclusion of antimicrobials tested has varied somewhat over the years. If only antimicrobials that have been tested all years are included, 11-17% of the isolates were resistant to three of more antimicrobials. Among these, nearly half were resistant to four antimicrobials or more (5-8%), with resistance to streptomycin, ampicillin, tetracycline and trimethoprim-sulphonamides being by far the most common trait (Table Dog III.). One isolate from 1995 was resistant to all included substances.

While the majority of the isolates showed full susceptibility or were resistant to at most two antimicrobials, the above shows that the remainder were often truly multiresistant. The comparatively high frequency of multiresistance probably reflects a high proportion of treatment failures and recurrent cases among the cases sampled. Resistance to all the drugs most commonly used to treat urinary tract infections, i.e. ampicillin, enrofloxacin and trimethoprim-sulphonamides were found in 2-5% of the isolates. All but one of these isolates were also resistant to streptomycin, and all but three to tetracycline. Thus, in some cases the choice of antimicrobials for treatment is severely limited, which emphasises the need for culture and subsequent testing for susceptibility as a basis for selection of antimicrobials for treatment of recurrent and non-responding urinary tract infections.

Table Dog II. Occurrence of resistance among *Escherichia coli* in dogs the years 1992-93, 1995, 2000, 2001 and 2002 and distribution of MICs for the isolates from 2002. All isolates are from diagnostic submissions of urine samples. Bold vertical lines indicate break-point for resistance

Substance	Percent resistant					Distribution (%) of MICs[1] 2002 (mg/L)									
	1992-93 n=150	1995 n=96	2000 n=186	2001 n=183	2002 n=204	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	19	24	20	19	18				7.4	61.3	12.3	1.0	18.1		
Chloramphenicol	5	9	5	2[5]	-										
Enrofloxacin	9	9	12	9	8	1.0	91.2	2.9	2.5	2.5					
Gentamicin	1	2	3	4	<1					78.4	19.6	1.5		0.5	
Neomycin	8	7	6	4[3]	-										
Nitrofurantoin	2	3	2	2	<1								97.1	2.0	1.0
Streptomycin	16	28	17	16	14						4.4	36.8	41.7	3.4	13.7
Tetracycline	17	22	13	11[4]	11				29.4	48.5	7.8	2.9	11.3		
Trim-Sulpha[2]	9	12	12	12	12[5]			82.8	2.5	2.0	1.0	11.8			

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); [3] 137 isolates tested; [4] 181 isolates tested; [5] 203 isolates tested.

Table Dog III. Resistance patterns of isolates of *E. coli* resistant to four or more antimicrobials Annual number of isolates with each pattern, where "R" in hatched fields denote resistance. Only antimicrobials that have been tested all selected years are included

1992-93 (n=150)	1995 (n=96)	2000 (n=186)	2001 (n=183)	2002 (n=204)	Total (n=819)	Resistance pattern[1]						
						Sm	Am	T-S	Tc	Gm	Ef	Ni
	1				1	R	R	R	R	R	R	R
			3		3	R	R	R	R	R	R	S
1					1	R	R	R	R	S	R	R
			2		2	R	R	R	R	R	S	S
3	3	6	4	4	20	R	R	R	R	S	R	S
				1	1	R	R	R	R	S	S	R
3	3	4	3	6	19	R	R	R	R	S	S	S
			1		1	R	R	R	S	R	S	R
		1			1	R	R	R	S	S	R	S
		1		1	2	R	R	S	R	S	R	S
	1				1	R	S	R	R	S	S	R
1		2		1	4	R	S	S	R	R	R	S
		1	2		3	S	R	R	R	S	R	S
8 (5%)	8 (8%)	15 (8%)	15 (8%)	13 (6%)	59 (7%)							

[1] Sm: streptomycin; Am: ampicillin; T-S: trimethoprim-sulfonamide; Tc: tetracycline; Gm: gentamicin; Ef: enrofloxacin; Ni: nitrofurantoin.

Cat

Isolates included

Antimicrobial susceptibility in *Escherichia coli* isolated from urine is presented. All isolates emanate from diagnostic submissions and might include repeat isolates from the same patients. Isolates from cats in the central-eastern part of Sweden are probably over-represented. Further, it is likely that there is a bias towards isolates from cats with recurrent disease or from therapeutic failures. The number of isolates investigated per year is small, but has increased somewhat over the time period studied (Table Cat I). It can therefore not be excluded that the criteria for submission have changed, and any inferences on trends must be made with caution.

Results and comments

Escherichia coli

Figures on resistance in feline pathogens in Sweden have not been presented previously. Levels of resistance to ampicillin, streptomycin, tetracycline, the combination trimethoprim-sulphonamides and fluoroquinolones are of the same magnitude as for dogs (10-20%) (Tables Cat I and Dog II). The levels of resistance to tetracycline in isolates from cats were notably lower in 2002 compared to previous years. Levels of resistance to fluoroquinolones (enrofloxacin) have increased gradually over the period studies and were by 2002 as high as 15%. The breakpoint chosen for this report is based on microbiological criteria. If a higher breakpoint is applied, >1 mg/L, the levels of resistance for 1992-1997, 1998-2000, 2001 and 2002 are 2, 7, 8 and 9%, respectively. The antimicrobials most commonly prescribed for cats are beta-lactams (ampicillin, amoxicillin and penicillin-V), tetracyclines and fluoroquinolones (Odensvik *et al*, 2001). The latest available figures on use in pets are from 1998, when about 9 700 prescriptions for fluoroquinolones intended for cats were dispensed at Swedish pharmacies. No reliable figures on demographics of cats in Sweden are available, so the population exposure is difficult to assess. However, considering the comparatively high frequency of resistance to fluoroquinolones, close monitoring of both use and levels of resistance seems warranted.

As for dogs, the inclusion of antimicrobials tested has varied somewhat over the years. If only antimicrobials that have been tested all years are included, 11-16% of the isolates were multiresistant, and 4-8% were resistant to four or more of the antimicrobials included. No temporal trends could be observed. The most common pattern with resistance to four or more antimicrobials included resistance to streptomycin, ampicillin, tetracycline and trimethoprim-sulphonamide (2-5%), and these isolates were mostly also resistant to fluoroquinolones. Combined resistance to ampicillin, trimethoprim-sulfonamides and enrofloxacin, the drugs most commonly used to treat urinary tract infections, occurred in 2-8% of the isolates. The level of multiresistance, and the patterns observed, is similar to what is described for *E. coli* isolated from dogs (see above). Considering the small number of isolates submitted for susceptibility tests, the material is likely to be biased towards recurrent cases or treatment failures. Notwithstanding, the observed high levels of resistance and multiresistance show that in such cases, the choice of antimicrobials for treatment may be severely limited and must be based on culture and susceptibility tests.

Table Cat I. Occurrence of resistance among *Escherichia coli* in cats the years 1992-97, 1998-00, 2001 and 2002 and distribution of MICs for the isolates from 2002. All isolates are from diagnostic submissions of urine samples. Bold vertical lines indicate breakpoint for resistance

Substance	Percent resistant				Distribution (%) of MICs[1] 2002 (mg/L)									
	1992-97 n=61	1998-00 n=74	2001 n=36	2002 n=46	≤0.12	0.25	0.5	1	2	4	8	16	32	>32
Ampicillin	26	34	31[4]	22				10.9	50.0	15.2	2.2	21.7		
Chloramphenicol	3	3	4[5]	-										
Enrofloxacin	5	8	11	15		84.8	2.2	4.3	8.7					
Gentamicin	0	3	8	4					80.4	15.2			4.3	
Neomycin	0	3	8[5]	-										
Nitrofurantoin	2	2	0[4]	7[6]								93.3		6.7
Streptomycin	25	18	14	20							50.0	28.3	2.2	19.6
Tetracycline	28	16	19	9				34.8	43.5	8.7	4.3	8.7		
Trim-Sulpha[2]	7	10[3]	14[4]	11			80.4	2.2	6.5		10.9			

[1] Hatched fields denote range of dilutions tested for each substance. MICs above the range are given as the concentration closest to the range. MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; [2] Concentration of trimethoprim given, tested in concentration ratio 1/20 (trimethoprim/sulphamethoxazole); [4] 73 isolates tested; [4] 35 isolates tested; [5] 26 isolates tested; [6] 45 isolates tested.

Appendix 1: Demographic data

Statistics on animal numbers and agricultural holdings with animals are provided by Statistics Sweden in collaboration with the Board of Agriculture. Figures are based either on total census or on samples of the populations. The counting is made in June and/or December. Statistics is published annually as a Yearbook of Agricultural Statistics and also on the Internet via the websites for Statistics Sweden (www.scb.se) or the Board of Agriculture (www.sjv.se). Specific sources are given in footnotes to the tables below.

The number of animals and holdings counted at the time of census are given in Table AP1 I and II, and the number of animals slaughtered on an annual basis is given in Table AP1 III. In addition, the volume slaughtered (expressed in tonnes) is given in Table AP1 IV.

The total number of food producing animals in Sweden has decreased notably over the last two decades, and the herd size has increased. In June 2002, the number of dairy cows had decreased by 11% from 1995 (Table AP1 I), but the herd average size had increased by 36% and is today 37 cows. Likewise, the herd size has increased markedly in pig production. Between 1995 and 2002, the number of pigs slaughtered decreased by 12% (Table AP1 I) but the average herd size for fattening pigs increased by 114% (today 336 pigs). The production of chickens for slaughter has almost doubled from 1980 until 2002 (Table AP1 III).

Table AP1 I. Number of livestock (in thousands) from 1980-2002[1] . The figures represent census figures from counts of all, or samples of the populations in the given years

	1980	1985	1990	1995	2000[4]	2001[4]	2002[4]
Cattle							
Dairy cows	*656*	*646*	*576*	*482*	*428*	*418*	*417*
Beef cows	*71*	*59*	*75*	*157*	*167*	*166*	*169*
Other cattle > 1 year	*614*	*570*	*544*	*596*	*589*	*573*	*553*
Calves < 1 year	*595*	*563*	*524*	*542*	*500*	*494*	*498*
Total, cattle	1 935	1 837	1 718	1 777	1 685	1651	1 637
Swine							
Boars & sows	*290*	*260*	*230*	*245*	*206*	*216*	*211*
Fattening pigs >20 kg [2]	*1 254*	*1 127*	*1 025*	*1 300*	*1 146*	*1 090*	*1 096*
Piglets <20kg [3]	*1 170*	*1 113*	*1 009*	*769*	*566*	*586*	*574*
Total, swine	2 714	2 500	2 264	2 313	1 918	1 892	1 881
Sheep							
Ewes and rams	*161*	*173*	*161*	*195*	*198*	*208*	*198*
Lambs	*231*	*252*	*244*	*266*	*234*	*244*	*229*
Total, sheep	392	425	405	462	432	452	427
Laying hens							
Hens	*5 937*	*6 548*	*6 392*	*6 100*	*5 670*	*5 687*	*4 732*
Chickens reared for laying	*2 636*	*2 159*	*2 176*	*1 812*	*1 654*	*1 721*	*1 537*
Total, hens	8 573	8 708	8 568	7 912	7 324	7 408	6 269

[1] Source: Yearbook of Agricultural Statistics, Sweden 1981, 1986, 1991, 1996, 2001, 2002 and Statistical Messages, JO 20 SM 0202. For 1980 and 1985 only cattle and sheep at premises with more than 2 ha counted; [2] Before 1995, the figure denotes pigs above 3 months of age; [3] Before 1995, the figure denotes pigs below 3 months of age; [4] The numbers are based on countings made in June 2000, 2001 and 2002.

Table AP1 II. Number of holdings with animals of different types from 1980-2002[1]

Animal species	1980	1985	1990	1995	2000	2001	2002
Cattle							
Dairy cows	*44 100*	*30 100*	*25 900*	*17 700*	*12 700*	*11 800*	*11 300*
Beef cows	*12 400*	*10 300*	*10 900*	*17 100*	*13 900*	*13 600*	*13 100*
Other cattle >1 year	*63 200*	*52 700*	*42 700*	*39 200*	*30 500*	*29 100*	*27 800*
Calves <1 year	*62 300*	*52 000*	*42 000*	*36 500*	*27 700*	*26 300*	*25 200*
Sheep, excluding lambs	10 100	10 500	9 700	10 000	8 000	8 100	7 400
Swine	26 100	19 900	14 300	10 800	4 800	4 500	2 700
Laying hens	23 600	17 500	12 900	9 600	5 700	5 800	5 300
Chickens reared for laying	5 100	2 700	1 900	1 400	700	1 000	800

[1]Source: Yearbooks of Agricultural Statistics, Sweden 1981, 1986, 1991, 1996, 2002 and Statistical Messages, JO 20 SM 0202.

Table AP1 III. Number of animals slaughtered (in thousands) at slaughterhouses from 1980-2002[1]

Animal species	1980	1985	1990	1995	2000	2001	2002
Cattle							
Cattle >1 year	*574*	*584*	*523*	*502*	*490*	*463*	*472*
Calves < 1 year	*130*	*138*	*70*	*46*	*39*	*34*	*34*
Total, cattle	704	722	593	548	529	497	506
Swine	4 153	4 283	3 659	3 763	3 251	3 198	3 282
Sheep	302	328	280	145	202	197	197
Chickens (broiler)	40 466	36 410	38 577	60 300	68 617	73 350	77 383

[1] Sources: For 1980-1995: Yearbooks of Agricultural Statistics, Sweden 1981, 1986, 1991 and 1996 except for chickens where figures were supplied by the National Food Administration, and for 2000-2002 Statistical messages JO 48 SM 0302 (all animal species).

Table AP1 IV. Quantity of livestock slaughtered (in tonnes) at slaughterhouses during 1990-2002[1]

Animal species	1990	1995	2000	2001	2002
Cattle					
Cattle >1 year	*138.4*	*140.1*	*145.4*	*139.1*	*142.3*
Calves < 1 year	*5.4*	*3.2*	*4.4*	*4.1*	*4.2*
Total, cattle	143.8	143.3	149.8	143.2	146.5
Swine	289.2	308.8	277.0	275.9	283.8
Sheep	4.9	3.5	3.9	3.8	3.9
Chickens (broiler)	44.0	74.2	89.9	96.1	101.4

[1] Sources: For years 1990 and 1995 Yearbooks of Agricultural Statistics, Sweden 1991 and 1996 except for chickens where figures were supplied by the National Food administration, and for 2000-2002 Statistical messages JO 48 SM 0302 (all animal species).

Appendix 2: Materials and methods, use of antimicrobials

Wholesaler data

Antimicrobial drugs used in veterinary medicine in Sweden are only available on veterinary prescription. Furthermore, antimicrobial drugs have to be dispensed through pharmacies, which in turn are supplied solely by two drug wholesalers. Sales statistics are available from Apoteket AB (The National Corporation of Swedish Pharmacies).

These statistics describe the amount of medicinal products sold from the wholesalers to the pharmacies. As the pharmacies stock a limited number of veterinary drugs, the wholesalers´statistics can be used as an approximation on the actual usage of antimicrobials. Wholesalers´ data have a very high degree of completeness. This is explained by the fact that the wholesalers represent the entire drug distribution network, i.e., there are no other sources of antimicrobials for use or prescription by veterinarians.

Sweden has a long tradition in drug consumption statistics. Apoteket AB, former Apoteksbolaget AB, has since 1976 monitored the consumption of drugs for use in humans mainly by using wholesalers´ statistics. In the case of drugs for animal use, SVA and Apoteket AB have collaborated over the years and data on the total use of antimicrobials for animals in Sweden are available since 1980. For a review of the figures from 1980-2000 as well as references to publications on which that review is based, see SVARM 2000. From 2003, Apoteket AB has the formal responsibility to gather such data. Further, the Board of Agriculture has recently been appointed competent governmental authority and will, from 2006, report statistics per animal species (food producing animals).

Classification of drugs

Veterinary medicinal drugs are classified according to the Anatomical Therapeutic Chemical veterinary classification system (ATCvet) (WHO, Guidelines for ATCvet classification). The system is based on the same main principles as the ATC classification system for substances used in human medicine. In both the ATC and ATCvet systems, drugs are divided into groups according to their therapeutic use. First, they are divided into 15 anatomical groups, classified as QA-QV in the ATCvet system (without Q in the system for human drugs), on basis of their main therapeutic use. Thereafter subdivision is made according to therapeutic main groups, which is followed by a further division in chemical/therapeutic subgroups.

Antimicrobials are classified in the QJ group - general anti-infectives for systemic use. However, antimicrobials can also be found in other groups such as QA (alimentary tract and metabolism), QD (dermatologicals), QG (genito-urinary system) and QS (sensory organs) depending on the therapeutic use.

Inclusion criteria

All veterinary antibacterial drugs authorised for use in animals except dermatologicals, ophtalmologicals and otologicals were included (i.e., ATCvet codes QA, QG and QJ). Veterinary drugs are preparations authorised for use in animals. Human drugs may be authorised not only for humans, but for animals as well. This latter category is not included in the statistics. However, no such drugs are authorised for use in the major food producing animal species, and the volume sold is very limited.

Drugs with antibacterial activity can also be found in other groups, notably among the antiprotozoals (QP51). Of these, the nitroimidazoles were included earlier but no such substances are presently authorised for use in animals. Sulfaclozine is licensed for treament of coccidiosis only and has therefore not been included. The ionophoric antibiotics are presently regulated as feed additives and not sold though pharmacies and are therefore not included in the wholesalers' statistics. However, the Board of agriculture 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.

Units of measurement for feed

On basis of knowledge on how different drugs are or have been used, a selection of products that have only or mostly been used for treatment of groups of pigs was made. Group treatment of calves is not common practice in Sweden. Very small amounts are used for poultry (SVARM 2000). Hence, it can be assumed that the bulk of the sales of drugs for group treatment from 1988 and onwards was intended for treatment of enteric and respiratory infections of pigs. Before 1986, antimicrobial growth promoters were used both for chickens and pigs. For pigs, the quinoxalines (olaquindox, carbadox) were by far the most widely used. Thus, from this group only the quinoxalines were included. Further, it was assumed that most of the sales of macrolides, tetracyclines and nitroimidazoles were intended for use in pigs also before 1988.

The figures are given as kg active substance, as g active substance per pig slaughtered and as corresponding kg feed per pig slaughtered. For the latter two measures, figures on number of pigs slaughtered were taken from Statistics Sweden (see Appendix 1). The figures on kg active substance were calculated to corresponding kg of feed by use of doses suggested by Beskow (cited and used in Wierup *et al*, 1987), or the authorised dose as given in the Swedish Compendium of Veterinary Drugs (FASS VET., 2002). The doses used for calculation are given in Table AC IV.

Distribution of veterinary medicines in Sweden

Marketing of drugs in Sweden is regulated by the Medicinal Products Act, which applies both to human and veterinary drugs. According to the Act, a medicinal product may not be sold until it has been granted marketing authorisation by the Medical Products Agency (MPA). The MPA has issued provisions concerning authorisation, distribution and prescription of veterinary medicinal products.

The state-owned Apoteket AB has exclusive rights regarding retail sales of medicines in Sweden. Apoteket AB operates according to guidelines set out in an agreement with the State. According to the Act only pharmacies run by Apoteket AB are permitted to sell drugs. This implies that veterinarians in Sweden are not permitted to sell drugs, although they may for practical reasons hand over medicines for emergency use. Veterinarians are, however, under no conditions permitted to make a profit from dispensing medicines.

Appendix 3: Materials and methods, resistance monitoring

Sampling strategy

Zoonotic bacteria

Salmonella

Isolates of *Salmonella* from warm-blooded animals (wild and domesticated) are included. Salmonellosis in animals is a notifiable disease in Sweden. It is mandatory that at least one isolate from each notified incident, including incidents detected in the Swedish *Salmonella* control programme, is confirmed at SVA. The first isolate from each animal species in each notified incident is included in the material presented in SVARM. Therefore, the material is thought to be representative for *Salmonella* prevalent among animals in Sweden.

Campylobacter

Campylobacter spp. was isolated from cloacal swabs from healthy broiler chickens sampled at slaughter as part of a Swedish *Campylobacter* programme started in 2001. From a total of 1016 flocks, 104 isolates, each representing one flock, were randomly selected for susceptibility testing. The selection was stratified by spring and fall. The isolates were stored in -70°C until testing. At subculture, 4 isolates did not grow and consequently, 100 isolates were finally included.

Indicator bacteria

Indicator bacteria, *Escherichia coli* and *Enterococcus* spp., were isolated from samples of intestinal content (caecum) from healthy broiler chickens. Samples were collected at slaughter.

Four abattoirs for chickens participated in the collection of samples. These abattoirs are geographically separated and accounted for 69 percent of the total slaughter in Sweden during 2001. The number of samples collected at each abattoir was proportional to the respective annual slaughter volume.

Sampling was performed weekly, with exceptions for holidays and summer vacations, by meat inspection staff or abattoir personnel. Each sample collected from chickens represents a unique flock, but not necessarily a unique production site. By these measures, bacterial isolates included are from randomly selected healthy individuals of Swedish flocks.

Animal pathogens

Isolates of animal pathogens included, except mastitis pathogens from dairy cows, emanate from routine bacteriological examinations of clinical submissions or post-mortem examinations at SVA. Mastitis pathogens included are the first hundred isolates of the respective bacterial species obtained in a project years 2002-03 where milk samples from cases of acute mastitis were collected by practicing veterinarians. The number of samples collected in each region of the country was proportional to the number of dairy cows in the region.

Isolates included from pigs are *E. coli* from the gastrointestinal tract (gut content, faecal samples or mesenteric lymph nodes) and *Brachyspira hyodysenteriae* isolated from faecal samples. From cattle, *E. coli* from the gastro-intestinal tract (gut content, faecal samples or mesenteric lymph nodes) and *Staphyloccoccus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* isolated in milk from dairy cows with acute mastitis are included. From horses, *Streptococcus zooepidemicus* from the respiratory tract and *E. coli* from the genital tract of mares are included. From dogs and cats *E. coli* isolated from samples of urine are included and from dogs also *Staphylococcus intermedius* isolated from skin samples.

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 NMKL (NMKL Nr 71, 1999). Confirmatory identification and serotyping of isolates was performed at the Department of Bacteriology, SVA following to standard procedures according to Kaufmann and White. The Dept. of Bacteriology, SVA is accredited for isolation, identification and serotyping of *Salmonella*. Phagetyping of *S.* Typhimurium and *S.* Enteritidis was performed by Swedish Institute for Infectious Disease Control (SMI), Stockholm using the Colindale scheme.

Campylobacter

Campylobacter spp. from chickens was isolated and identified at SVA according to standard procedures. Samples were cultured for thermophilic *Campylobacter* spp. using a modified NMKL method (NMKL Nr 119, 1990) using Preston enrichment broth and Preston selective agar, and incubation at 42°C. Identification was based on colony morphology, microscopic appearance including motility and the following phenotypic characteristics: production of oxidase, catalase and hippurate hydrolysis reaction (Nachamkin, 1999). With these tests, hippurate-positive *C. jejuni* can be identified whereas other isolates are described as hippurate-negative thermophilic *Campylobacter* spp. The Dept. of Bacteriology, SVA is accredited for isolation and identification of *Campylobacter* spp.

Indicator bacteria

Escherichia coli

Approximately 0.5 g of intestinal content from chicken caeca was diluted in 4.5 mL phosphate buffered saline (PBS, pH 7.2). After thorough mixing, 0.1 mL of this suspension was spread on MacConkey agar. After incubation overnight at 37°C, one lactose positive colony with morphology typical for *E. coli* was sub-cultivated on horseblood agar (5% v/v) and the isolate was subsequently tested for production of tryptofanase (indole) and ß-glucuronidase (p-nitrophenyl-ß-D- glucopyranosiduronic acid, PGUA). Only lactose-positive isolates with typical morphology and positive reactions in both tests were selected for susceptibility tests.

Enterococci

Intestinal content from chickens (caecum) was diluted as described for *E. coli* and cultured both on solid media without selective antibiotics and in enrichment broth supplemented with vancomycin (8 mg/L) or ampicillin (16 mg/L).

Culture without selective antibiotics: Of the diluted faecal material, 0.1 mL was spread onto Slanetz-Bartley (SlaBa) agar and incubated for 48 h at 37°C. One colony, randomly chosen, was 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 tested for antimicrobial susceptibility and identified to species level according to Devriese *et al.* (1993) by use of the following biochemical tests: mannitol, sorbitol, arabinose, saccharose, ribose, methyl-alfa-D-glucopyranoside and raffinose.

Enrichment in broth with vancomycin: Approximately 0.5 g of intestinal content from chickens (caecum) was added to 4.5 mL enrichment broth (Enterococcosel) supplemented with 8 mg/L vancomycin and incubated in 37°C for 24 hours. After incubation in 37°C for 24 h, 0.1 mL of the enriched culture was spread on SlaBa agar supplemented with 8 mg/L vancomycin and incubated in 37°C for 48 h. From plates showing growth of colonies typical for enterococci, at least one colony of each morphological type was sub-cultivated on bile-esculin agar and blood agar (37°C, for 24 h). Colonies with typical morphology on blood agar and positive reaction on bile-esculin agar were confirmed as enterococci by a test for pyrrolidonyl arylamidase (PYR) and were further identified to species level as above. All isolates identified to *Enterococcus* spp were selected for antimicrobial susceptibility and identified to species level.

Enterococcal isolates with MICs of vancomycin above >32 mg/L were subtyped with the PhenePlate™ system (PhPlate Microplate Techniques AB, Stockholm, Sweden). Mostly, the PhP RF, were the fingerprinting is based on the kinetics of 11 biochemical reactions, was used (Kühn *et al.*, 1995). In addition, selected isolates were genotyped with PCR allowing confirmation of identification as *E. faecium* and *E. faecalis*, and identification of the *vanA*- and *vanB*-genes (Dutka-Malen *et al.*, 1995).

Enrichment in broth with ampicillin: During the first half of 2002, all samples from chickens were also enriched in Enterococcosel broth with ampicillin (16 mg/L). The procedures were as described for enrichment in broth with vancomycin, except that the enriched broth culture was subcultured to SlaBa with ampicillin (16 mg/L).

Animal pathogens

Animal pathogens, except udder pathogens, were isolated and identified at the Dept. of Bacteriology, SVA with accredited methodology, following standard procedures. Udder pathogens were isolated by the practising veterinarians collecting the milk samples. Subsequently, the original culture plates were sent to the Dept. of Mastitis, SVA for final bacteriological diagnosis by use of accredited methodology, following standard procedures.

Susceptibility testing

All antimicrobial susceptibility tests were performed using dilution methods. For bacteria other than *Campylobacter* spp and *Brachyspira* spp, the tests were performed following the standards for microdilution of the National Committee of Clinical Laboratory Standards (NCCLS, 1999) in Mueller-Hinton broth. The microdilution panels used, VetMIC™, are produced at the Dept. of Antibiotics, SVA. Different panels were used depending on the bacterial species tested and the original purpose of the investigation (monitoring or clinical diagnostics; see Table AP3 I).

For *Campylobacter* spp and *Brachyspira* spp, there are currently no accepted standards for broth dilution susceptibility tests. The microdilution method described by NCCLS was adapted for *Campylobacter* spp. VetMIC™ panels were used, and each well was inoculated with 100 µl Mueller-Hinton broth with an inoculum density of approximately 10^6 CFU/ml. The panels were incubated in 37°C for 48 hours in a microaerophilic atmosphere.

For susceptibility testing of *Brachyspira hyodysenteriae*, a macrobroth dilution method with a specially developed VetMIC™ panel 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% fetal calf serum ($1x10^6$-$5x10^6$ CFU/ml). The trays were incubated in an anaerobic atmosphere for four days on a shaker.

Minimum inhibitory concentration (MIC) was recorded as the lowest concentration of the antimicrobial that inhibits bacterial growth. An isolate was regarded as resistant to a specific antimicrobial when its MIC was distinctly higher than those of inherently susceptible strains of the bacterial species in question. In other words, microbiological criteria were used to define resistance. Where appropriate, the breakpoints suggested by NCCLS (1999) for animal pathogens were also taken into consideration. The breakpoints used for defining resistance are shown in Table AP3 I.

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).

Quality assurance system

The Dept. of Antibiotics, Dept. of Bacteriology and laboratories at SVA using VetMIC™ for antimicrobial susceptibility tests, are accredited to perform the method according to SS-EN ISO/IEC 17025 by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC). Likewise, the laboratories responsible for isolation and identification of animal pathogens and zoonotic bacteria (Dept. of Bacteriology and Dept. of Mastitis) are accredited for these procedures according to the same standard.

For susceptibility tests of zoonotic and indicator bacteria, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* CCUG 11284 (analogue to *Campylobacter jejuni* ATCC 33560) were included as quality controls at least on a weekly basis. Relevant control strains were also included and evaluated at least once weekly for animal pathogens.

The Dept. of Antibiotics participates in several proficiency tests for antimicrobial susceptibility testing. These are arranged either as national or international studies. Likewise, the Dept of Bacteriology and the Dept. of Mastitis participate 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 routinely registered in an Oracle database at SVA. From this, relevant data were extracted to an Access database.

Data on samples for cultivation of indicator bacteria were recorded in an Access database on arrival of samples. Recorded data were animal species, date of sampling, abattoir and herd of origin. For samples from chickens, flock of origin was also recorded. For *Campylobacter,* only isolate identity and animal species were initially registered. Subsequently, results of laboratory investigations were recorded in the same database.

Calculations and analysis of data were performed in the computer programs Access, Excel and Minitab.

Concerning confidence limits

When the prevalence of antimicrobial resistance is close to zero, e.g. when one out of 120 isolates are 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.

There are several ways out of the dilemma; one 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 intervals (Rao, 1965). Using this approach, the confidence interval 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.

Table AP3 I. Breakpoints (mg/L) used for antimicrobial susceptibility testing of bacteria. Isolates with MIC values higher than the given figures are considered resistant

Antimicrobial agent	*Salmonella enterica*	*E. coli* (indicator)	*E. coli* (pathogen; pig)	*E. coli* (pathogen; cattle, horse)	*E. coli* (pathogen; dog, cat)	Enterococci (indicator)	*Streptococcus zooepidemicus*	*Streptococcus dysgalactiae* *Streptococcus uberis*	*Staphylococcus intermedius*	*Staphylococcus aureus*	*Brachyspira hyodysenteriae*	*Campylobacter*
Amoxicillin & clavulanic acid[1]	>8	>8										
Ampicillin	>8	>8	>8	>8	>8	>8	>8					>16
Apramycin	>32	>32										
Avilamycin						>16		>16		>16		
Bacitracin[2]						>32						
Ceftiofur	>2	>2	>2	>2			>2					
Cephalothin	>16							>1	>2	>1		
Chloramphenicol	>16	>16	>16	>16	>16		>8	>8	>16	>16		
Clindamycin							>4	>4	>4	>4		
Enrofloxacin	>0.25	>0.25	>0.25	>0.25	>0.25				>0.5			>1
Erythromycin						>4	>4	>2	>4	>2		>16
Flavomycin						>32						
Florfenicol	>16	>16	>16	>16			>16					
Gentamicin	>8	>8	>8	>8	>8	>512			>4	>4		>8
Nalidixic acid	>16	>16										>16
Narasin						>2						
Neomycin	>8	>8	>16	>8	>8	>1024			>32	>32		
Nitrofurantoin			>32	>32	>32				>32			
Oxacillin									>1	>2		
Penicillin							>1	>0.25	[4]	[4]		
Spiramycin							>16	>16	>16	>32		
Streptomycin	>32	>32	>32	>32	>32	>1024				>32		
Sulphamethoxazole	>256	>256										
Tetracycline	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8		>8
Tiamulin											>2	
Trimethoprim	>8	>8										
Trimethoprim & sulfamethoxazole[3]	>0.5		>4	>4	>4		>4	>4	>2	>2		
Tylosin											>16	
Vancomycin						>16		>16		>16		
Virginiamycin						>8		>4		>4		

[1] Concentration of amoxicillin given, tested with clavulanic acid in concentration ratio 2/1; [2] MIC in U/mL; [3] Concentration of trimethoprim given, tested with sulfamethoxazole in concentration ration 1/20; [4] ß-lactamase production.

Appendix 4: Antimicrobial agents licensed

Antimicrobial agents licensed for therapy in veterinary medicine in Sweden year 2002 are listed in Table AP4 I. Only substances licensed for systemic, oral, intrauterine or intramammary use are included (ATCvet codes QJ, QG, QA and QP). Data from FASS VET. 2002. For explanation of ATCvet code, see Appendix 2.

Table AP4 I. Antimicrobial agents authorised for therapeutic use in cattle, pigs, poultry, horses, dogs and cats in Sweden, 2002. Routes of administration are indicated[1].

Antimicrobial agent	ATCvet code	Animal species					
		Cattle	Pigs	Poultry	Horses	Dogs	Cats
Tetracyclines							
Doxycycline	QJ01A A02		O			O	O
Oxytetracycline	QJ01A A06, QG51A A01	I O U	I O U	O		O	O
Beta-lactams, penicillins							
Ampicillin	QJ01C A01	O	O		O	O	O
Amoxicillin	QJ01C A04		I			I O	O
Penicillin G	QJ01C E01	I	I		I		
Penicillin G, procaine	QJ01C E09	I	I		I	I	I
Penicillin V	QJ01C E02					O	O
Amoxicillin/Clavulanic acid	QJ01C R02		I			I O	I O
Beta-lactams, cephalosporins							
Cephalexin	QJ01D A01					O	
Cefadroxil	QJ01D A09					O	O
Ceftiofur	QJ01D A90	I					
Sulphonamides/Trimethoprim							
Sulphadiazine/Trimethoprim	QJ01E W10	I	I		I O	O	O
Sulphadoxine/Trimethoprim	QJ01E W13	I	I		I		
Sulphonamides							
Formosulphatiazole	QA07A B90	O	O		O	O	O
Sulphaclozin	QP51A G04			O			
Macrolides							
Spiramycin	QJ01F A02	I					
Tylosin	QJ01F A90	I	I O	O		I	I
Lincosamides							
Clindamycin	QJ01F F01					O	O
Pirlimycin	QJ51F F90	M					
Aminoglycosides							
Gentamicin	QJ01G B03				I U	I	I
Dihydrostreptomycin (DHS)	QA07A A90	O U	O U		O U	O	O
Fluoroquinolones							
Enrofloxacin	QJ01M A90	I	I	O		I O	I O
Danofloxacin	QJ01M A92	I	I				
Marbofloxacin	QJ01M A93					O	O
Orbifloxacin	QJ01M A95					O	
Pleuromutilins							
Tiamulin	QJ01X X92		I O				
Combinations							
Penicillin G, procaine/DHS	QJ01R A01, QJ51R C23	I M	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					

[1] O = oral; I = injection; U = intrauterine; M = intramammary.

Appendix 5: References

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