MARAN 2007

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
In 2006/2007

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Colophon

This report is published under the acronym MARAN-2007 by VANTURES, the Veterinary Antibiotic Usage and Resistance Surveillance Working Group. The information presented in MARAN-2007 is based on a collation of data from ongoing surveillance systems on the use of antimicrobial agents in animal husbandry and the development of antimicrobial resistance in bacteria of animal origin and of relevance to public health.
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Summary and Conclusions

Usage of antibiotics

The use of veterinary medicines may lead to risks concerning human health, environment, food safety, animal health and animal welfare. For example the use of antibiotics leads to the development of resistance in bacteria. The policy of the Dutch government, c.q. the Ministry of Agriculture, Nature and Food Quality, is aimed at a reduction of antibiotic resistance levels in bacteria, for instance by implementing policies to achieve a reduction of the use of antibiotics by farmers. A clear insight in the use of veterinary medicines is needed to be able to deal with this matter properly.

The Ministry of Agriculture, Nature and Food Quality (LNV) requested LEI to study the use of antibiotics on Dutch livestock farms in 2007. The objective of this study was to determine the use of antibiotics in 2007 and compare the use with that in previous years. In addition, the study searched for reasons for the extent to which farms make use of antibiotics.

The analyses in this report are based on the information collected in LEI's Farm Accountancy Data Network, as well as data from FIDIN and antibiotic use figures from other countries.

The analysis of the FIDIN figures reveals that the therapeutic veterinary antibiotic use (including antimicrobial growth promoters) has increased in the period 1999-2007 by 83% and the growth promoters have been banned, first partly and as from 2006 entirely. The use increased by 8.9% in 2007 as compared to 2006. The therapeutic antibiotic use per kg live weight in 2007 was twice as high as in 1999. A part of this increase may be accounted for by a substitution of growth promoters. In comparison with other countries for which veterinary antibiotic consumption figures are available, the antibiotic use per average food-producing animal is greatest in the Netherlands. However, it is not yet clear whether this is applicable to all or a number of sectors. Antibiotic use is increasing in the Netherlands, Denmark and Germany, while the use is stable in the other five countries examined during this study.

Figure 1 shows the antibiotic use at a sample of 159 farms in 2007. The four vertical lines in the figure indicate the confidence interval, i.e. on the basis of this sample the average antibiotic use in the Netherlands can be stated to lie within the upper and lower limits with 95% confidence.

Figure 2 shows the changes in the use at all farm categories in the sentinel farms during the years 2004-2007. The antibiotic use for fattening pigs and, in particular, broilers, exhibits an evident increase. The increase for fattening pigs is statistically significant and numbers 3.5 daily dosages per animal year and will lie within an increase of 2.0 to 11.5 daily dosages per animal year with 95% confidence. Also the increase for broilers is statistically significant and numbers 13.8 daily dosages per animal year and will lie within an increase of 4.1 to 27.0 daily dosages per animal year with 95% confidence[1].

1 The confidence limits for the increase 2004-2007 were calculated based on unweighted data.

Figure S1 Average number of daily dosages per animal year in 2007, with the corresponding 95% confidence intervals.

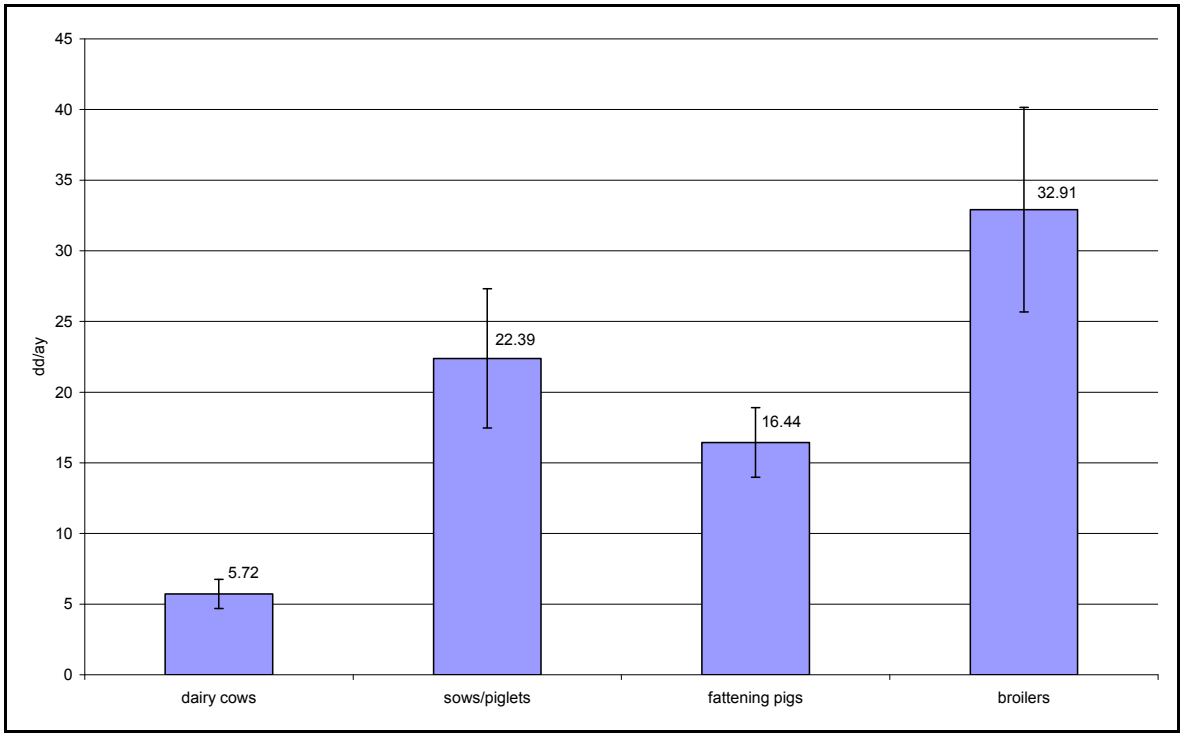
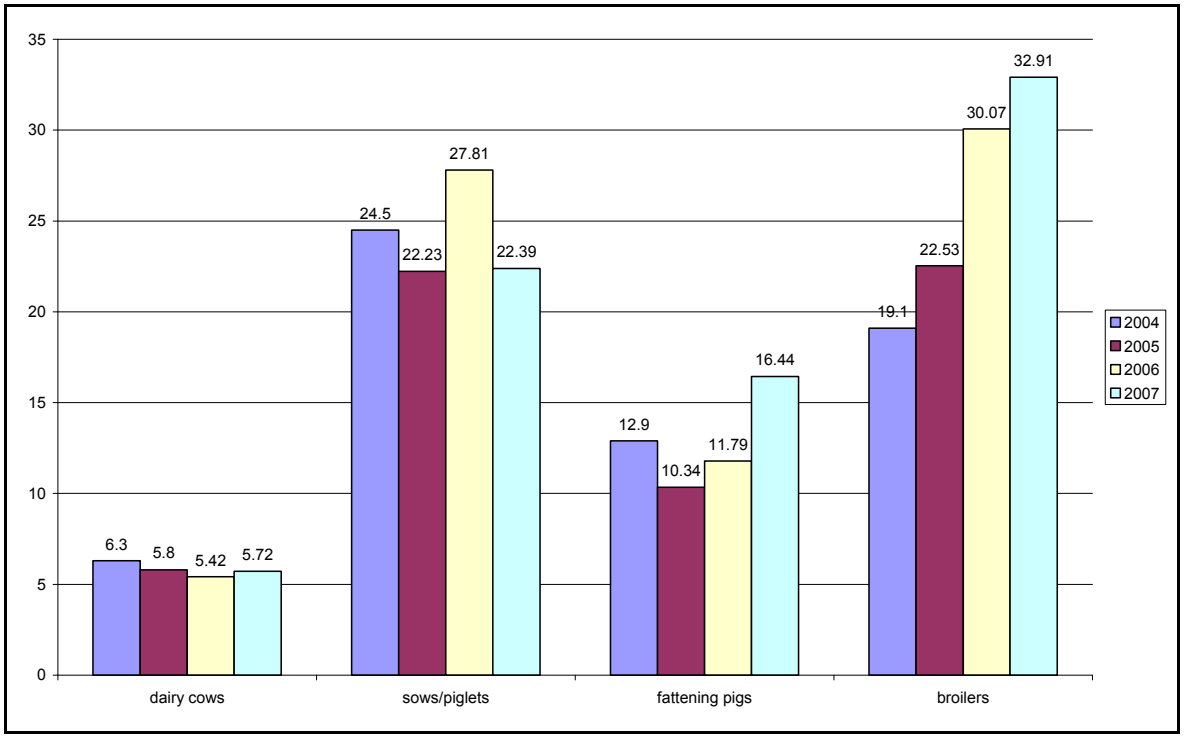


Figure S2 Trend in antibiotic usage from 2004 to 2007, expressed as daily dosages per animal year



Antibiotic use has remained virtually unchanged at the dairy cattle farms. The proportion of antibiotics administered via the udder (intramammary) fell from 3.5 daily dosages per animal year (dd/ay) in 2006 to 2.9 dd/ay in 2007, whereby the use of cloxacillin decreased and the use of amoxicillin-clavulanic acid increased.

At the fattening pig farms the total use of antibiotics has increased. The use of tetracyclines decreased (–1.3 dd/ay) whilst the use of other antibiotics increased (in particular, colistin sulphate, +3 dd/ay) and macrolides (+1 dd/ay).

The antibiotic use at the farms with sows/piglets in 2007 was comparable to that in 2006, both in terms of the total use and the use of the various groups of antibiotics. A further analysis of sow farms revealed that: a) in general, antibiotic use is higher at farms with more sows; b) farms that occasionally make preventive use of antibiotics have the lowest average antibiotic use, whilst farms that routinely give preventive treatments have the highest average use and the use at farms that never give preventive treatments lies in between the two; c) the sow holders' assessment of the health of their animals is related to their use of antibiotics: antibiotic use was lower at farms where the holders were of the opinion that their animals were in good health.

The total antibiotic use increased at broiler farms, largely due to the increased use of penicillins (+3.9 dd/ay; amoxicillin and phenoxymethylpenicillin). Fluoroquinolones and aminoglycosides are primarily used in the broiler sector, although the use of fluoroquinolones has decreased (-1.8 dd/ay). Antibiotic use varies greatly between farms: the 25% of the farms that use most antibiotics account for 49% of the total antibiotic use.

Farms in all sectors that have used a large amount of antibiotics in a given year in many cases also used large amounts of antibiotics in the previous year. Consequently, antibiotic use at many farms is relatively stable.

Trends in resistance

In 2006/2007 *S*. Typhimurium and *S*. Enteritidis were the most prevalent serovars in humans, third was the antigenic variant *S*. *enterica* subspecies *enterica* 1,4,5,12:i:-, a monophasic variant of *S*. Typhimurium which is emerging internationally since 2004. Pigs and cattle were the most important animal sources of *S*. Typhimurium. Layers (eggs) and foreign travel were the most important sources for *S*. Enteritidis. In broilers *S*. Java was isolated most frequently. In broilers *S*. Enteritidis and *S*. Typhimurium constituted only a small fraction of all salmonella's.

In 2006/2007, 81 cefotaxime resistant, ESBL suspected strains were found, which was substantially more than in 2004/2005 (n = 21). These isolates belonged predominantly to the serovar *S*. Java (63%), of which only 1 strain was isolated from a human source, the others all from poultry sources. Resistance to cefotaxime is increasing at an alarming rate. The isolates from humans originate partly from exotic sources, but also from poultry. In animals poultry is up to now the only reservoir for ESBL-producing salmonella's.

In *C. jejuni* resistance levels for ciprofloxacin and nalidixic acid were substantially higher than in previous years, in particular in broilers. At the same time in human isolates a substantial increase in resistance to fluoroquinolones was observed as well. In 2007 for the first time an erythromycin resistant *C. jejuni* strain was isolated from a cow. Erythromycin resistance is however a regular finding in humans to some extent travel related, or related to consumption of contaminated imported products, or due to human therapeutic use of macrolides. In *C. coli* resistance to macrolides occurred frequently. In 2006/2007 for the first time next to erythromycin, the related antibiotics clarithromycin and tulathromycin were included in the tests. The resistance levels were slightly different from erythromycin, which is probably due to the cut-off values used.

Resistance in *E. coli* O157 was traditionally rarely present. In human isolates resistance levels were low. In isolates from calves however, resistance was quite commonly present, with levels varying from 0.7 – 20.7 %. In calves one ESBL suspected isolate was detected.

Overall, the resistance levels of food-borne commensal *E. coli* were highest in broilers and poultry raw meat products, followed by veal calves, slaughter pigs and dairy cattle, in which resistance is rare. In broilers and slaughter pigs, for most antibiotics the resistance levels show a tendency to increase. This increase is also obvious for multi-drug resistance. In broilers and poultry raw meat products resistance to cefotaxime indicative of extended spectrum beta-lactamases (ESBLs), has increased from 9.7% in 2004 to almost 17% in 2006/2007.

For the enterococci as indicator organisms, highest resistance levels were observed for tetracycline and erythromycin in both bacterial species. In broiler chickens the resistance levels show a tendency to decrease, while in the other animals the levels seem to be stable. Resistance to vancomycin remains present but is rare. Multi drug resistance is very common in veal calves, pigs and broilers, but not in dairy cows

In bovine respiratory disease pathogens the resistance levels were higher in *Mannheimia haemolytica*. Tetracycline resistance occurred most frequently in both species, although substantially more frequently in *M. haemolytica*, in which species also resistance to amoxicilline, flumequine and chloramphenicol occurred frequently. Resistance to aminoglycosides was present in single isolates in *Pasteurella multocida* only. Single isolates resistant to tilmicosin and florfenicol were detected. For florfenicol this was the first time a resistant PMU is reported in The Netherlands.

The resistance levels for *E. coli* strains isolated from milk samples from cows suffering from mastitis were low except for ampicillin. In 2006/2007 the first ESBL-producing *E. coli*'s were isolated from mastitis. The coliform bacteria showed a high level of resistance to ampicillin and to the combination with clavulanic acid. All isolates were susceptible to cefoperazone and cefquinome. The *S. aureus* isolates tested were susceptible to most antibiotics. 9.2% were penicillin resistant. In 2006/2007 one *S. aureus* was identified to be MRSA. The strain belonged to the animal associated clonal complex 398. The coagulase negative staphylococci were more resistant than *S. aureus.* 56% were resistant to penicillin and 1.5% to oxacillin (mecA-positive).

Conclusions

It can be concluded that therapeutic usage of antibiotics in food animals in The Netherlands has almost doubled in the past decade. Likely determinants for the increase are the ban of the growth promoters and the up scaling of farm sizes. The quality of animal feed is under stress because of high prices on the global market and the ban of animal protein in feed, which may affect the digestibility in the GI-tract which is compensated by oral antibiotics**.** The continuous increase in antibiotic usage is striking because currently there is an intense debate about the negative effects and public health risks of antibiotic usage in intensive animal husbandry. The resistance levels in animal bacteria show a simultaneous tendency to increase, both for individual drugs and multi-drug resistance.

Next to the frequent occurrence of MRSA in Dutch food-animals, of particular concern is the rapid increase in the occurrence in ESBL-producing organisms in predominantly poultry and poultry meat products. This increase is both observed to occur frequently in the commensal GI-tract flora of broiler chickens, in *Salmonella* serovars from broilers and to a lesser extend in *Salmonella* from humans. Compared to the animal derived MRSA the risks of acquiring ESBLs by humans are different. MRSA is mainly transmitted by direct contact, while for the ESBLs food borne transmission is likely to contribute to the dissemination, since the genes are located on mobile genetic element and therefore transferable within and between bacterial species.

As a result of the concerns for public health related to antibiotic usage in intensive animal husbandry, the animal production sectors have signed a convenant in 2008 with the purpose to minimize resistance by responsible use of antibiotics. Each sector has developed a detailed plan to implement this. The major aspects of the plans are:

- To define the responsibilities for prescribing, selling and administration of antibiotics
- To increase the transparency by:
    - implementing a control system on these agreements
    - monitoring of antibiotic usage at different levels
- To implement research and communication strategies for responsible antibiotic usage

These are important developments; however, these plans will only be effective for prevention of current and future resistance development if the result will be a substantial reduction in the exposure of animals to antibiotics.

Samenvatting en Conclusies

Gebruik van antibiotica

Het LEI heeft in opdracht van het ministerie van LNV onderzoek gedaan naar het gebruik van antibiotica in de Nederlandse veehouderij in 2007. Het doel van dit onderzoek is het bepalen van het antibioticagebruik in 2007 en de vergelijking met voorgaande jaren. Daarnaast is gezocht naar oorzaken van de mate waarin antibiotica werd gebruikt op de bedrijven.

Voor de analyses in dit rapport is gebruik gemaakt van informatie verzameld binnen het Bedrijven Informatienet van het LEI. Daarnaast is gebruik gemaakt van gegevens van FIDIN en van antibiotica gebruikscijfers van andere landen.

Uit de analyse van de FIDIN cijfers blijkt dat het totale therapeutisch veterinaire antibioticagebruik in Nederland sinds 1999 met 83% is toegenomen, dat is een toename van gemiddeld 7,9% per jaar. In 2007 blijkt het gebruik ten opzichte van 2006 met 8,9% te zijn toegenomen. Het therapeutisch antibioticagebruik per kg levend gewicht is ten opzichte van 1999 verdubbeld.

Nederland past vergeleken met de andere landen waarvan verbruikscijfers bekend zijn, veterinair per gemiddeld aanwezig dier het meeste antibiotica toe. Echter, voor specifieke diersectoren kan deze vergelijking anders liggen. Tevens stijgt het antibioticagebruik van Nederland momenteel elk jaar, terwijl van de andere vergeleken landen alleen Denemarken en Duitsland een stijging laten zien en de overige vijf landen een stabiel gebruik hebben.

Figuur S1 (zie blz. .7) laat het antibioticagebruik in dagdoseringen van 2007 zien met het bijbehorende 95% betrouwbaarheidsinterval, op de 159 steekproefbedrijven.

Figuur S2 (zie blz. 7) laat het verloop zien van alle bedrijven uit de steekproef van 2004 tot en met 2007. Alleen de vleesvarkens en vooral de vleeskuikens laten een duidelijk stijgende lijn zien. De toename voor vleesvarkens in deze periode is statistisch significant en bedraagt 3,5 dagdoseringen per dierjaar en zal met 95% zekerheid liggen tussen een toename van 2,0 tot 11,5 dagdoseringen per dierjaar. Ook de toename voor vleeskuikens is statistisch significant, bedraagt 13,8 dagdoseringen per dierjaar en zal met 95% zekerheid liggen tussen een toename van 4,1 en 27,0 dagdoseringen per dierjaar. Per achtereenvolgend jaar bekeken zijn de stijgingen niet significant.

Het percentage antibiotica dat bij melkvee intramammair wordt toegediend is afgenomen van 64% in 2006 naar 53% in 2007. Binnen de intramammair toegediende antibiotica is een afname te zien in het gebruik van cloxacilline en een toename in het gebruik van amoxicilline-clavulaanzuur.

Opvallend bij het antibioticagebruik op vleesvarkensbedrijven is de afname van het gebruik van tetracyclines (-1,3) en de toename van het gebruik van overige antibiotica (colistinesulfaat) (+3) en macroliden (+1).

Het antibioticagebruik bij zeugen /biggen laat over 2007 eenzelfde beeld zien als over 2006, zowel in totaal gebruik, als het gebruik verdeeld over de verschillende antibioticagroepen. Uit de nadere analyse op zeugenbedrijven is gebleken dat: a) op zeugenbedrijven een sterk verband is tussen bedrijfsomvang en antibioticagebruik, hoe groter het bedrijf, des te meer antibiotica-gebruik, waarbij het gemiddelde antibioticagebruik per bedrijf het laagst is bij bedrijven die soms preventief antibiotica gebruiken en het hoogst bij bedrijven die altijd preventief antibiotica gebruiken; b) er een significant verschil bestaat tussen het gemiddelde antibioticagebruik van de groepen van bedrijven die altijd, soms of nooit preventief antibiotica gebruiken; c) de inschatting van zeugenhouders over de gezondheidstoestand van hun dieren een voorspeller kan zijn voor de mate van het antibioticagebruik; d) een betere schatting wordt verkregen van het werkelijk antibioticagebruik als per middel het gebruik kan worden toegewezen aan het juiste varken (zeugen of biggen).

In de steekproef is de vleeskuikensector de enige sector die quinolonen en aminoglycosiden gebruikt. Het totale antibioticagebruik in deze sector is toegenomen. De toename is vooral terug te vinden bij het gebruik van penicillines (+3,9). Het gebruik van quinolonen is afgenomen (-1,8). Er zijn grote verschillen in antibioticagebruik tussen bedrijven. 25% van de bedrijven, is verantwoordelijk voor 49% van het antibioticagebruik.

In alle sectoren geldt dat bedrijven die in een jaar veel antibiotica hebben gebruikt, dat vaak in het voorgaande jaar ook al deden. Veel bedrijven zijn dus vrij stabiel in de mate waarin ze antibiotica toepassen.

Trends in resistentie

In 2006/2007 *S*. Typhimurium and *S*. Enteritidis waren de meest prevalente serotypen in humane infecties en op de derde plaats kwam de antigene variant *S. enterica* subspecies *enterica* 1,4,5,12:i:-, een monofasische variant van *S*. Typhimurium die in toenemende mate voorkomt sinds 2004. Varkens en rundvee waren de belangrijkste dierlijke bronnen van *S*. Typhimurium. Legkippen (eieren) en reizen naar het buitenland waren de belangrijkste bronnen voor *S*. Enteritidis. In vleeskuikens kwam *S*. Java het meest voor en vormen *S*. Enteritidis en *S*. Typhimurium slechts een kleine fractie van alle salmonella's.
In 2006/2007 werden 81 cefotaxim resistente, ESBL verdachte salmonella's gevonden, wat een beduidende toename is ten opzichte van 2004/2005 (n = 21). Deze isolaten behoorden vooral tot het serotype *S*. Java (63%), van welke slechts 1 stam afkomstig was van een humane bron. De overige werden allen in pluimvee geïsoleerd**.** Resistentie tegen cefotaxim neemt alarmerend toe. De isolaten uit mensen stamden deels van exotische bronnen door reizen naar het verre Oosten of Afrika, maar deels ook van pluimvee. In dieren is tot nu toe pluimvee het enige reservoir voor ESBL-producerende salmonella's.

In *C. jejuni* waren de resistentie niveaus voor ciprofloxacin en nalidixinezuur beduidend hoger dan in voorgaande jaren, en vooral in vleeskuikens**.** Tegelijkertijd werd ook in humane isolaten een beduidende toename in resistentie tegen fluoroquinolonen gezien. In 2007 werd voor de eerste keer een erythromycine resistente *C. jejuni* geïsoleerd uit een rund. Erythromycine resistentie is echter een regelmatige bevinding in mensen, wat deels wordt veroorzaakt door reizen, door consumptie van gecontamineerde geïmporteerde producten, of als gevolg van humane therapeutisch gebruik van macroliden. In *C. coli* kwam resistentie tegen de macroliden vaak voor. In 2006/2007 werden voor de eerste keer naast erythromycine, de verwante antibiotica clarithromycine en tulathromycine meegetest. The resistentieniveaus verschillen licht van erythromycine, wat waarschijnlijk komt door de gebruikte afkapwaarden.

In *E. coli* O157 komt resistentie slechts zelden voor. In humane isolaten waren de resistentieniveaus laag. Echter, in kalverisolaten kwam resistentie relatief vaak voor, met waarden variërend van 0.7 – 20.7 %. In kalveren werd ook één ESBL-verdacht isolaat gevonden.

Over het geheel genomen waren de resistentieniveaus van commensale *E. coli* als indicatororganisme het hoogst in vleeskuikens en rauw vlees van pluimvee, gevolgd door vleeskalveren, vleesvarkens en melkvee. In vleeskuikens en vleesvarkens, vertonen de resistentieniveaus toenemende tendensen voor de meeste antibiotica. Dit gebeurt ook voor multiresistentie. In vleeskuikens (en pluimveevlees) is het voorkomen van resistentie tegen cefotaxim, wat indicatief is voor de aanwezigheid van extended spectrum beta-lactamasen (ESBL's), toegenomen van 9.7% in 2004 tot bijna 17% in 2006/2007.

Voor de enterokokken als indicatororganismen, werden de hoogste resistentieniveaus gezien voor tetracycline en erythromycine**.** In vleeskuikens vertonen de resistentieniveaus een toenemende trend terwijl ze in de andere diersoorten stabiel lijken. Resistentie tegen vancomycine komt nog steeds incidenteel voor. Multiresistentie komt algemeen voor in vleeskalveren, vleesvarkens en vleeskuikens, maar niet n melkkoeien.

In bovine luchtwegpathogenen waren de resistentieniveaus hoger in *Mannheimia haemolytica* dan in *Pasteurella multocida*. Tetracycline resistentie kwam het vaakst voor in beide species, maar beduidend vaker in *M. haemolytica*, in welke species ook resistentie tegen amoxicilline, flumequine and chloramphenicol vaak voorkwam. Resistentie tegen aminoglycosiden kwam alleen voor in enkele *P. multocida* isolaten. Enkele isolaten die resistent waren tegen tilmicosin en florfenicol werden gedetecteerd. Voor florfenicol was dit de eerste keer dat er een resistente *P. multocida* is gerapporteerd in Nederland.

De resistentieniveaus van *E. coli* isolaten uit melkmonsters van mastitiskoeien waren laag met uitzondering van ampicillin. In 2006/2007 werd ook uit een mastitismonster de eerste ESBL-producerende *E. coli*'s geïsoleerd**.**

De coliforme bacteriën uit mastitismelk vertoonden hoge resistentieniveaus voor ampicilline en de combinatie met clavulaanzuur. Alle isolaten waren gevoelig voor cefoperazone en cefquinome.
De *S. aureus* isolaten uit mastitismelk waren gevoelig voor de meeste antibiotica. 9.2% was penicilline resistent. In 2006/2007werd één *S. aureus* uit mastitismelk geïdentificeerd als MRSA. De stam behoorde tot het diergeassocieerde clonal complex 398, wat ook bij varkens en kalveren veel voorkomt. De coagulase negatieve stafylokokken waren resistenter dan *S. aureus.* 56% was resistent tegen penicilline en 1.5% tegen oxacilline (mecA-positief).

Conclusies
Er kan worden geconcludeerd dat het therapeutisch antibioticumgebruik in voedselproducerende dieren in Nederland in het afgelopen decennium bijna verdubbeld is. Waarschijnlijke determinanten voor deze toename zijn het verbod van de groeibevorderaars en de schaalvergroting van de bedrijven. De kwaliteit van het diervoeder staat bovendien onder druk door hoge prijzen van grondstoffen op de wereldmarkt en het verbod van dierlijk eiwit in diervoeders. Dit kan de verteerbaarheid van het voedsel in de darm negatief beïnvloeden, wat gecompenseerd wordt door gebruik van antibiotica**.** De continue toename van gebruik is des te opvallender omdat er momenteel een intensief debat plaatsvindt over de negatieve effecten en volksgezondheidsrisico's van het gebruik inde dierlijke productie. De resistentieniveaus nemen tegelijkertijd toe, zowel voor individuele antibiotica als multiresistentie.

Naast het veelvuldig voorkomen van MRSA in Nederlandse voedselproducerende dieren, is vooral de snelle toename van ESBL-producerende organismen, vooral in pluimvee en pluimveevlees een reden voor zorg. Deze toename wordt zowel gezien bij de commensale flora van het darmkanaal van vleeskuikens, in *Salmonella* uit vleeskuikens en worden in mindere mate ook in *Salmonella* uit de mens geïsoleerd. In vergelijking met de diergerelateerde MRSA is er een verschil in het risico van het verkrijgen van ESBLs. MRSA wordt voornamelijk overgedragen naar de mens via direct contact, terwijl voor de ESBLs de voedselketen waarschijnlijk een rol speelt in de verspreiding omdat de genen op mobiele genetische elementen liggen die overdraagbaar zijn binnen en tussen bacteriespecies.

Als gevolg van de aan het antibioticumgebruik in de intensieve veehouderij gebonden risico's voor de volksgezondheid hebben de dierhouderij sectoren in 2008 een convenant getekend met als doel het verminderen van resistentie door een verantwoord gebruik van antibiotica. Iedere sector heeft hiervoor een plan ontwikkeld, waarvan de hoofdpunten bestaan uit:
- Het afspreken van de verantwoordelijkheden voor het voorschrijven, leveren en toedienen van antibiotica tussen dierenarts, veehouder en dierlijke productie industrie.
- Dit moet een transparant proces worden door:
    - het invoeren van een controlesysteem op deze afspraken
    - monitoren van antibioticumgebruik op verschillende niveaus
- Het ontwikkelen van een onderzoeks-, en communicatiestrategie voor verantwoord gebruik

Dit zijn belangrijke ontwikkelingen, echter deze plannen zullen alleen effectief zijn in het voorkómen van huidige en toekomstige resistentieontwikkeling in de veehouderij indien ze leiden tot een substantiële reductie van de blootstelling van dieren aan antibiotica.

I Usage of antibiotics in animal husbandry in the Netherlands

1. Introduction

Problem definition
The extent to which antibiotics are used for veterinary purposes on food producing animals can contribute to public and animal health risks**.** It is an important determinant for the development of antibiotic resistance within the treated animal populations**.** This is also recognised by the European commission: the member states are required to monitor antimicrobial resistance in relation to public health. Within this context, the monitoring of antibiotic use is also important. This report contains information about the monitoring results in the Netherlands**.**

Recent developments
Various developments in the Netherlands may have had an impact on the veterinary use of antibiotics during the last decade. An increase in antibiotic use could be caused by the prohibition of the use of growth promoters as from 1 January 2006. In addition, a new Animal Medicines Act implemented on the basis of Directive 2004/28/EC lays down a new structure for channeling animal medicines**.** Putative causes for the increased antibiotic use are the lower feed quality in the Netherlands (in comparison to other countries) due to the prohibition on animal protein in feed, and the increases in scale in the livestock farming sector. New active antimicrobial ingredients have also been introduced for use in food producing animals.

Developments that should result in lower antibiotic use and an awareness of the need to limit the use of antibiotics, include the discovery of human patients infected with MRSA originating from livestock, the increasing concerns about the high and increasing use of antibiotics both inside and outside the agricultural sector and the response of the sector and the authorities to those concerns in the form of convenants designed to reduce antibiotic use.

Monitoring by FIDIN
In the Netherlands, FIDIN - the veterinary pharmaceutical industry - provides continuous reporting of antibiotic use (FIDIN, 2008). These reports are produced on a voluntary basis**.** The figures stated in the reports give an impression of the total number of kilograms of antibiotics (active ingredients) used in the Netherlands at the level of pharmacotherapeutic groups (the groups of active ingredients, such as tetracyclines and quinolones). The figures do not provide an insight into the use per type of animal, but for all types of animal.

Necessity of constant and detailed monitoring
The MARAN reports (Mevius *et al.*, 2006) published over a number of years reveal that although the total number of animals produced in the Netherlands is decreasing, the therapeutic use of antibiotics is increasing. It was decided to monitor antibiotic use in the various sectors continuously and in great detail to obtain an improved insight into the underlying factors that could explain this increase. This is achieved by the detailed monitoring of a stratified sample of Dutch farms**.** This study reviewed farms that supply data to LEI´s FADN (Farm Accountancy Data Network). In recent years, this Farm Accountancy Data Network has proven very useful in recording and reporting data about antibiotic use (and other animal medication).

Objective and result
The objective of this study is to obtain an insight into the use and trends in antibiotic use in livestock farming. To this end, the number and types of antibiotics used on pigs, broilers and dairy cattle each year is determined at a group of sample farms in the Netherlands**.** These annual reports provide a good insight into trends in antibiotic use per type of livestock farming. In 2010, the types of animals that are monitored will be expanded to include data about veal farms**.**

This study provides information about antibiotic use in the different types of animals in Dutch livestock farming, the various active ingredients, and the amounts that are used. Further research into the risk factors influencing antibiotic use is promoted improving the insights into antibiotic use. This study examined the relationship between antibiotic use at individual farms and a number of technical and economic key figures**.** Our report also includes a further analysis of a number of segments, and an analysis of FIDIN's overall-use figures**.**

The VANTURES Working Party will combine the results from this study with data about resistance per pathogen per type of animal and publish the information in the MARAN reports**.** This is in accordance with the mandate the Working Party received from the national coordinative Antibiotic Resistance Platform.

Effect
The collected figures give an insight into the Dutch livestock-farming sector's use of antibiotics**.** The Ministry of Agriculture, Nature and Food Quality will use the results from the study to provide the European Commission information about antibiotic use at the type of animal level. In addition, the usage data can play an important role in explaining trends in resistance that have become apparent. Trends in antibiotic use can also be used to measure the effect of policy. Moreover, the government's policy can take account of the underlying risk factors that have resulted in the use of antibiotics**.**

2. Materials and methods

The analyses in this report are based on the information collected in LEI's Farm Accountancy Data Network, as well as data from FIDIN and antibiotic use figures from other countries**.**

2.1 FIDIN antibiotic use figures and country comparisons

The FIDIN reports state the total number of kilograms of antibiotics (active ingredient) used in the Netherlands at the level of pharmacotherapeutic groups**.** The figures give insight into the use for all types of animals, but not for the individual animal species**.**

This LEI study relates the total antibiotic use published by FIDIN to the number of animals in the Dutch livestock farming sector (pigs, broilers, veal calves, cattle, and sheep). In total, the numbers of these animals result in an estimated number of kilograms of animal in a country. The national antibiotic use is then divided between the numbers of animal kilograms**.** This yields information about trends in the antibiotic use per kilogram of live animal weight over the years, and corrects for yearly fluctuations in the number of animals produced.

The country comparisons are based on Eurostat figures on animal numbers for different European countries[2]. The other data were obtained from Utrecht University's Faculty of Animal Health (Van Geijlswijk *et al.*, 2009). The analysis for the Netherlands based on the method used for the calculations using the data for 2006 is enclosed in Annex 4.

[2] The Dutch data used last year (2006) were obtained from LEI /Statistics Netherlands (CBS), agricultural and horticultural figures**.** Consequently, the absolute figures from previous reports can no longer be compared directly with the figures in this report. These have been included in the calculation to provide for a realistic comparison with countries with a large number of sheep.

2.2 Farms in the Farm Accountancy Data Network

The results in this study are based on data from a number of farms in LEI's Farm Accountancy Data Network. The Data Network contains a representative sample of around 1,500 agricultural and horticultural farms in the Netherlands (Vrolijk and Van der Veen, 2008). Records are made of the economic data and technical key figures of these farms. Every year a number of farms are replaced by other farms to ensure that the database of the Data Network remains representative for Dutch livestock farming. Extremely detailed records have been kept of the animal-medicine data at some of the farms since 1999. Records are kept of each individual animal medication and every veterinary service.

This report reviews the use in 2007, and is based on 159 farms in the Farm Accountancy Data Network, of which 36 were dairy farms, 52 fattening pig farms, 42 sow farms, and 29 broiler farms**.** Separate records are kept of the usage figures for sows and fattening pigs at closed pig farms**.** Annex 3 lists the precise figures for the farms and animals in the sample over the course of the years**.**

This study made use of data about technical and economic key figures and antibiotic use at the farms contained in the Farm Accountancy Data Network, supplemented with data from a brief questionnaire with questions on accommodation, feed and health (Annex 5). Since not all additional data was available for all farms, a number of analyses have been carried out on a smaller group of farms**.** When this is the case, the analysis is accompanied by a statement of the number of farms involved.

In some instances, the results are applicable to all farms in the sample for 2007, and reference is then made to the *total group* (159 farms). Reliable statements about an increase or decrease in use can be made only when the monitoring of the antibiotic use is based on the same farms that took part in both 2006 and 2007. Reference is then made to the *comparison group* (123 farms). The *comparison group* is used for comparisons of the antibiotic use in 2006 and 2007. Table 2.1 specifies the *total group* for 2007.

Table 2.1 Characteristics of the 159 farms in the sample for 2007 (total group)

2007	Number of farms	Average number of animals on each farm	Number of animals on smallest farm	Number of animals on largest farm
Dairy cattle	36	84	22	241
Pigs				
-fattening pigs	52	2,477	177	7,182
-sows(/piglets)	42	473	98	1,155
Broilers	29	67,000	11,000	244,000

2.3 Antibiotic use: use in grams and daily dosages per animal year

The antibiotic use was analysed using two methods: 1) an analysis of the quantities of active ingredients in grams; and 2) an analysis of the number of daily dosages per animal year.

In addition to the use figures in daily dosages, this report also includes figures for the use in grams since this information can be used for comparisons with other studies, where relevant. However, a comparison on the basis of daily dosages is a much better method, because it more accurately predicts the exposure of animals of different weights to antibiotics**.**

Quantity of active ingredient in grams

This is based on the quantities of active ingredients in the antibiotics used on the farms**.** The total amounts of active ingredients were determined for each farm, and were expressed in terms of grams of active ingredient per average animal present per year (per animal year). Expressing the use per animal year provides for comparisons of farms with different vacancy periods**.** All the grams are then totalled.

This is often the sole method available for reporting information about use, since no better data is available. The FIDIN reports are one example. (FIDIN, 2008)

Daily dosages

Antibiotics vary in their potency and pharmacokinetic properties[3], and this is manifested in the form of the varying dosages per kilogram of body weight. The unit daily dosage is suitable for calculating the total exposure to different antibiotics and, for example, making comparisons per group. Adopting this approach offers an opportunity to obtain an improved insight into the relationship with the existence of or trends in the development of resistance. Moreover, this unit conforms to international developments in this field and developments in the human sector. The broader implementation of records of this nature will also improve the feasibility of comparing the resultant data, for example antibiotic use in different EU member states in similar livestock systems**.**

The number of daily dosages per animal year was determined by calculating the total number of kilograms of animal (the treatable weight) that can be treated with each active ingredient. This was then divided by the total weight of the number of livestock in the country[4]. This assumes that the average treatment is administered to animals with an average weight. Adopting this approach provides for calculations and comparisons of the total antibiotic use on farms, even when different active ingredients are involved. More information is given in the daily dosages box, which also includes an example of a calculation.

This information can then be used to obtain an insight into the total antibiotic use for a specific category of animal (for example, fattening pigs) on a group of farms (for example, all pig farms with fattening pigs). This is also expressed in terms of an average number of daily dosages per animal year for fattening pigs**.**

Daily dosages

The amounts of different active ingredients cannot simply be totalled since the effectiveness and kinetics (and, consequently, the dosage prescription) varies between active ingredients**.** However, active ingredients can be compared and totalled once the active ingredient in each antibiotic preparation is expressed in terms of the *daily dosage*. The daily dosage is a measure of the number of milligrams of a specific active ingredient required to treat one kilogram of animal in one day with that antibiotic preparation, and is based on the recorded average dosage of a medicine for a specific type of animal. These daily dosages can be totalled to determine the total exposure to antibiotics**.** The daily dosages are specific to the type of animal, and have been defined for cattle, pigs and chicken. Consequently, antibiotic preparations used to treat several types of animal can be administered using a range of daily dosages, i.e. the daily dosage for each type of animal.

Example of a calculation of the daily dosage

For example, a farm with 150 fattening pigs with an average weight of 70.2 kg used 2 litres of antibiotic preparation X during the course of one year (40% of which consists of active ingredient a and the remainder of solvent and supplements) and 20 kg of antibiotic preparation Y (25% of which consists of active ingredient b). Antibiotic preparation X contains active ingredient a: the specified dose is 10 mg a day per kg animal weight. Antibiotic preparation Y contains active ingredient b: the specified dose is 50 mg a day per kg animal weight.

Antibiotic preparation X can be used to treat (2,000 * 40% * 1000)/10 = 80,000 kg animal weight. Antibiotic preparation Y can be used to treat (20,000 * 25% * 1,000)/50 = 100,000 kg animal weight. Consequently, the farm has used antibiotics for a total of 180,000 kg animal weight. The farm has an average of 150 fattening pigs per year, with a total weight of 10,530 kg. 180,000 kg were

[3] Differences in dosage are determined by differences in potency as well as differences in assimilation and differences in distribution throughout the body.

[4] This is the average weight of the treated animals (in kilograms per animal) multiplied by the average number of animals present on the farm per year.

treated in that year, equivalent to 180,000/10,530 = 17.1 daily dosages. Consequently, an average fattening pig[5] on the farm in that year was administered a prescribed dosage of antibiotics on 17.1 days**.** In this example the farm uses 17.1 daily dosages per animal year of antibiotic preparation X plus Y.

Animal weights
In an ideal situation, the number of daily dosages should be determined on the basis of the treated weight of the treated animals. However, the information that is available is insufficient for the determination of the exact weight of the animals at the time of the administration of the medicine, and for this reason the calculations use the average weight per animal during the period the animal is on the farm. The following average weights have been used: dairy cow 600 kg, broiler 1kg, fattening pig 70.2 kg, sow 220 kg, maiden gilt 107.5 kg, piglet 12.5 kg, breeding boar 350 kg (ASG, 2007). For sow farms, the weight of the average number of sows, gilts, piglets and breeding boars is totalled.

2.4 Statistical analysis

Analyses at the level of individual farms are required to obtain an insight into the risk factors for antibiotic use. The data collected in the Data Network offers an opportunity for these analyses**.** Beforehand correlation tables were made followed by appropriate (multiple) linear regressions[6] to examine the relationship between the size of the farm, the level of the technical results, and the antibiotic use (in number of daily dosages). These analyses were carried out using the software packages SPSS and Genstat.

3. Trends in antibiotic usage

3.1 Trends in the total antibiotic use in the Netherlands

Figure 3.1 shows the trends in the total therapeutic antibiotic use in the Netherlands**.** The figure was prepared from usage figures collated by FIDIN (FIDIN, 2008).

[5] This refers to a pig on the farm throughout the year: however, there is no such pig. This is a method which can be used to provide for comparisons of farms with different vacancy rates**.** For example, a farm has 2 herds of animals a year, both of which comprise 200 animals that remain on the farm for 5.5 months**.** The farm is vacant during the first and last week of the year, and for 2 weeks between the two herds. The calculations for this farm are based on an average of 183 animals present on the farm. When a farm is vacant for six months and has a herd of 200 animals for six months then the calculations are based on an average of 100 animals on the farm.
[6] In the event of two or more quantitative variables taking the nominal ordinal or linear character of the variables into consideration.

Figure 3.1 Veterinary therapeutic antibiotic use from 1999-2007 (FIDIN, 2008)

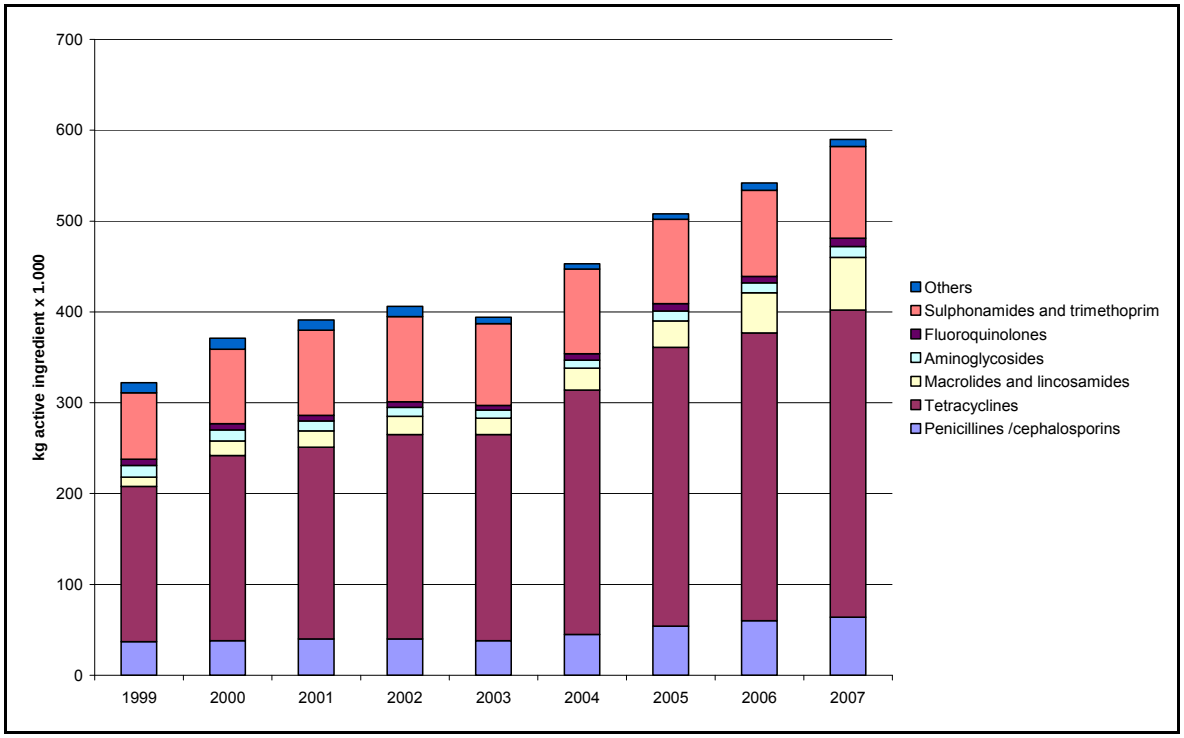


Figure 3.2 Total antibiotic use in the Netherlands, 1999 – 2007.

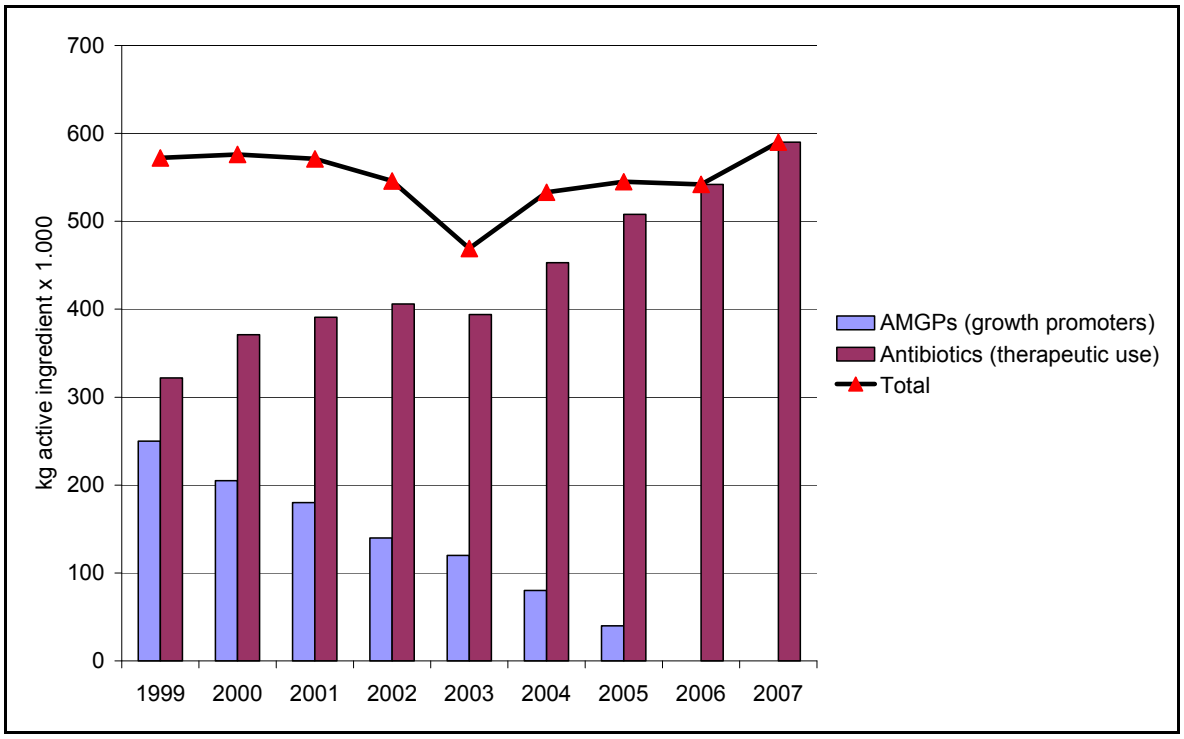
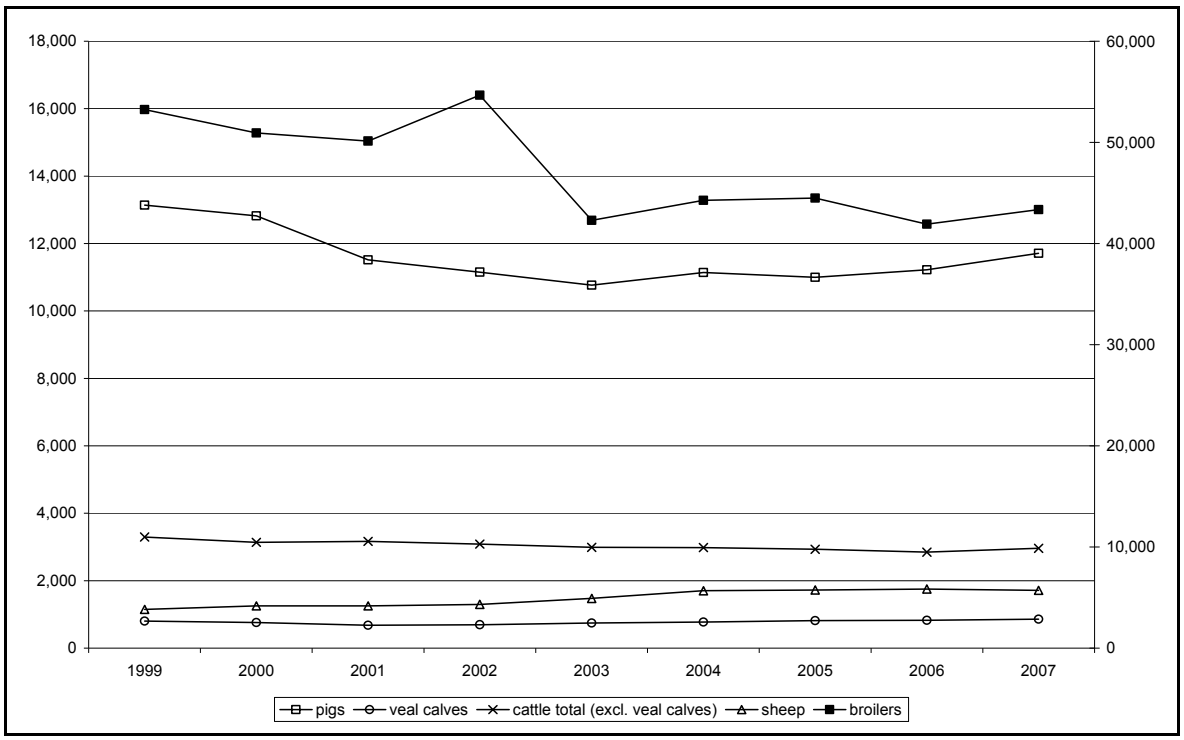


Figure 3.1 reveals that the total amount of antibiotics sold by the pharmaceutical industry in the Netherlands for therapeutic veterinary use has increased by 83% since 1998, equivalent to an average increase of 7.9% per year. The use increased by 8.9% in 2007 as compared to 2006.

The use of antimicrobial growth promoters (AMGP) was prohibited at the beginning of 2006. Figure 3.2 shows the trend in total antibiotic use including growth promoters**.** The total use increased by over 3% during the period from 1999 to 2007. The figures reveal an increase in total use during the period from 2003 through 2005 and a decline in the use of AMGPs**.** The total use (in kilograms) actually fell slightly in 2005 and 2006. In 2007, the use of antibiotics once again increased sharply as compared to 2006. A part of this increase may be accounted for by a substitution of growth promoters**.**

Over the years, the number of the livestock has also changed. The best possible insight into the trends in therapeutic antibiotic use is obtained by relating the total data in Figure 3.1 to the trends in the number of animals in the Netherlands. Figure 3.3 shows the trends in the numbers of animals**.**

Figure 3.3 Trends in livestock production in the Netherlands. Numbers of animals, 1999-2007 (x 1,000 animals). The modified scale on the right-hand axis indicates the numbers of broilers (solid squares).



Source: Eurostat 2008.

These yearly production numbers of animals are converted into live weight in Figure 3.4.. Although the number of broilers has fluctuated over the years, the resultant variation in the total animal weight (on average, 1 kg per broiler) is minimal. The other types of animal do not exhibit any significant differences**.** The higher live weights in 1999 and 2000 were due to the relatively larger number of pigs in those years**.**

In conclusion, the total antibiotic use is divided by the live weight present (in kg) to obtain the best possible insight into the actual trends in antibiotic use (see Figure 3.5. [7])

[7] The analysis of the data for 2006 presented last year was based on a different method of calculation. The effects of this are shown in Annex 4.

Figure 3.4 Trends in livestock in the Netherlands. Live weight, 1999-2007 (in millions of kg)

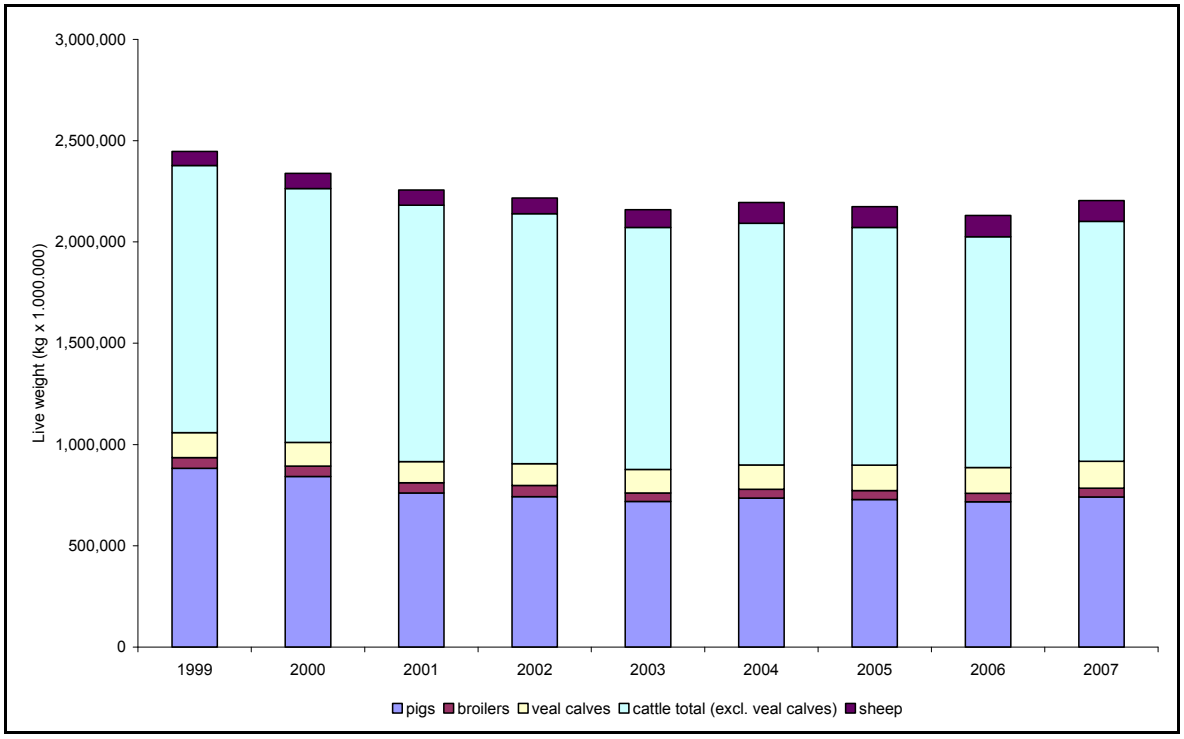


Figure 3.5 Total therapeutic antibiotic use 1999-2007, in mg per kg live weight

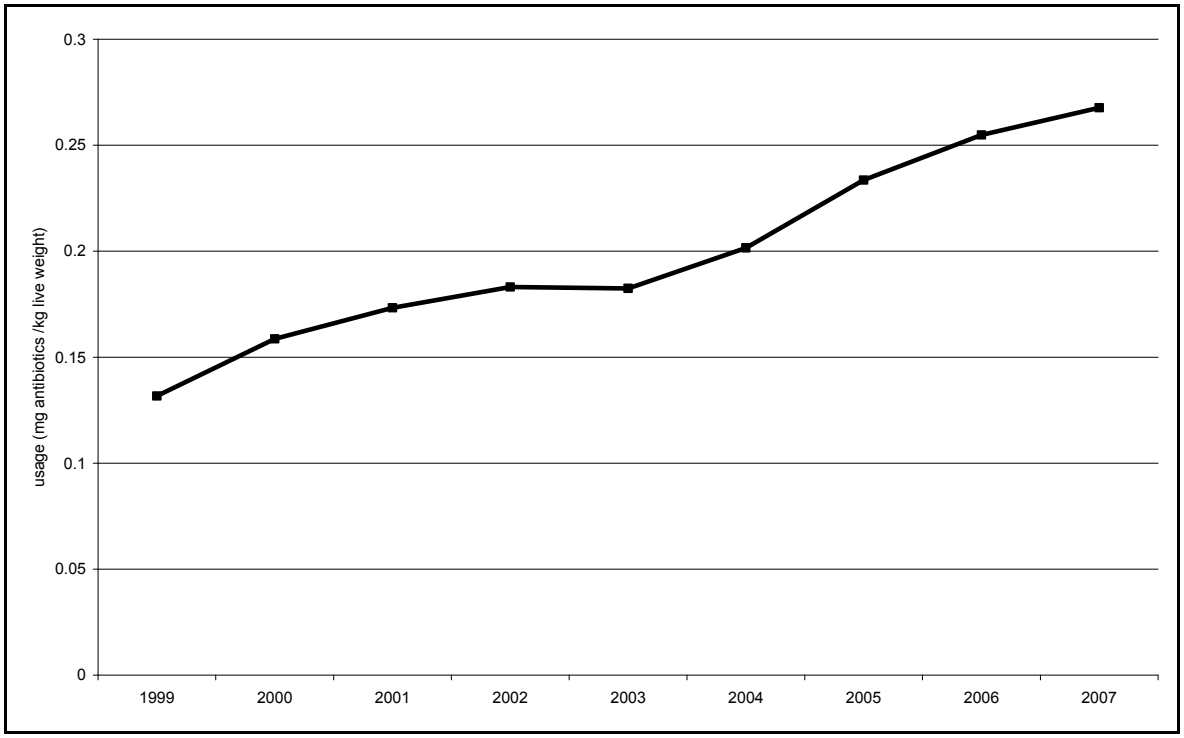


Figure 3.5 reveals that therapeutic antibiotic use expressed in terms of mg per kg live weight has doubled in the eight years since records began in 1999.

3.2 Usage outside the Netherlands[8]

Figures for the quantities of purchased or prescribed veterinary antibiotics have been published for a number of European countries. These countries also present the figures in terms of kg active ingredients. An insight into the volume and composition of antibiotic use in these countries can be obtained by collecting all the available data and converting the weight of active ingredients in each group of medicines into the treatable weight of animal (see Table 3.1). The total of these figures is related to the number of livestock in the relevant country.

Table 3.1 Conversion factors for kg antibiotic to treatable kg to daily dosage

Group of medicines	average dosage (mg/kg)	conversion factor, kg antibiotic to treatable kg
tetracyclines	8.22	121543
trim /sulfa combinations	23.0	43435
β-Lactams	7.09	141131
aminoglycosides	6.84	146196
macrolides	5.77	173441
fluoroquinolones	4.48	223012
other	5.00	199886

Figure 3.6. Daily dosages of antibiotics (calculated from the sold/delivered kg of active ingredient) per average animal per year in the various countries.

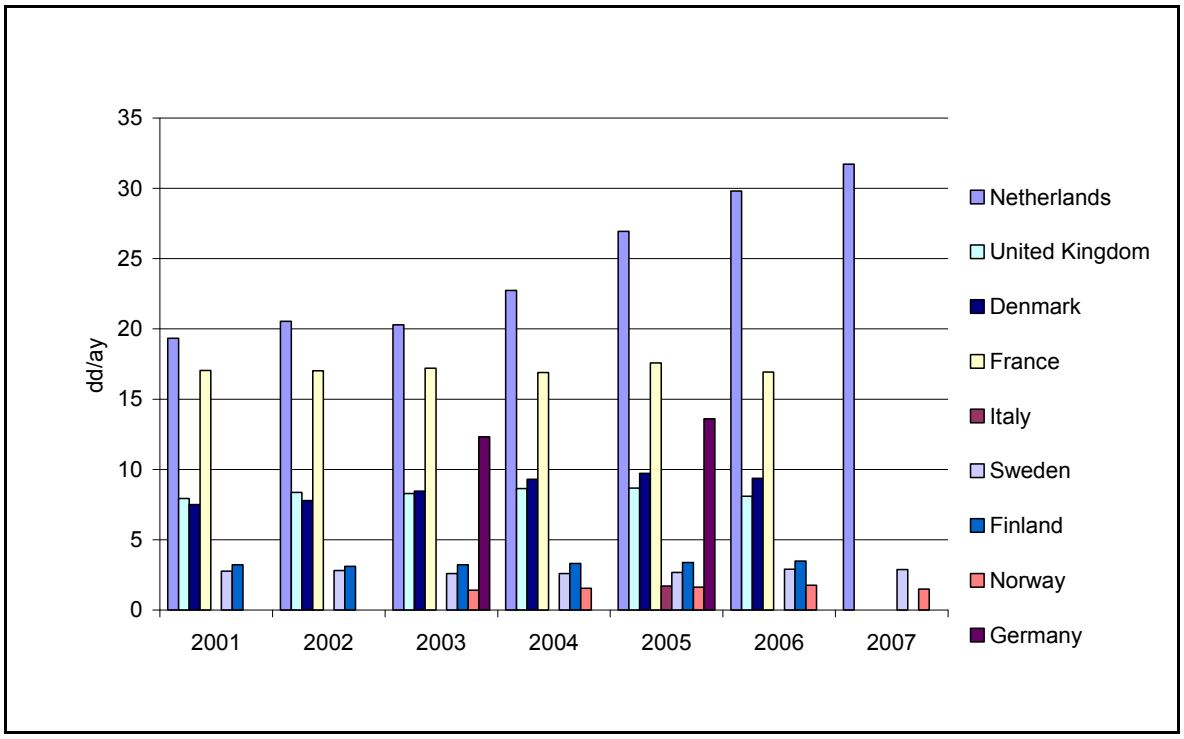


Figure 3.6 shows the calculated daily dosages of antibiotics that an average animal is administered per year in the various countries. The average animal in the Netherlands is administered a daily dosage of antibiotics on approximately 20 days (2001) to 30 days (2007) a year. The number of daily dosages is significantly lower in other countries, although the situation can differ for specific types of animals.

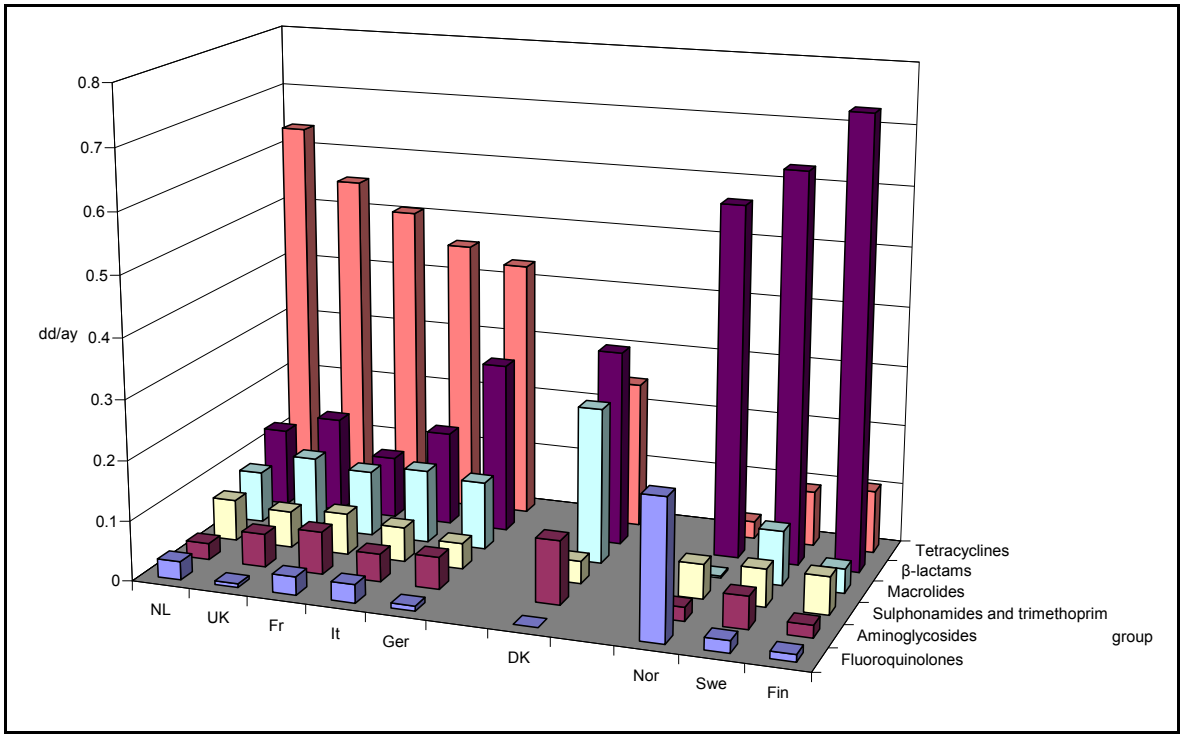
[8] This Subsection is, bar a few minor changes, reproduced from Van Geijlswijk *et al.* (2009).

The Dutch antibiotic use is currently increasing each year (cf. figures 3.1 and 3.5): use is also increasing in Germany and Denmark, whereas use in the other countries is relatively stable.

These differences are in part due to the major differences in the national animal populations (denominator data). France and the UK, for example, have large numbers of beef cattle and sheep that are always outdoors and receive very limited amounts of antibiotics. In the Netherlands, the use reflects the larger proportion of intensive livestock farming operations**.** Reports previously published in countries such as Denmark, where livestock farming exhibits more similarities with the Netherlands, indicate that antibiotic use is actually lower. Direct comparisons are complicated by the differences in the level at which records are kept: at a national sales level (Netherlands, France, Germany, UK, Finland, Norway) or at farm level (prescription level) (Denmark, Sweden [as from 2003]).

An overview of European antibiotic use by group of medicines gives an insight into the veterinary antibiotics policy pursued in the various countries**.** (see Figure 3.7)

Figure 3.7. Percentages (%) of the total antibiotic use (expressed as calculated daily dosages) of the six main groups of antimicrobial preparations in each country in 2005.



The following remarks can be made about the groups of medicines used in the various countries:

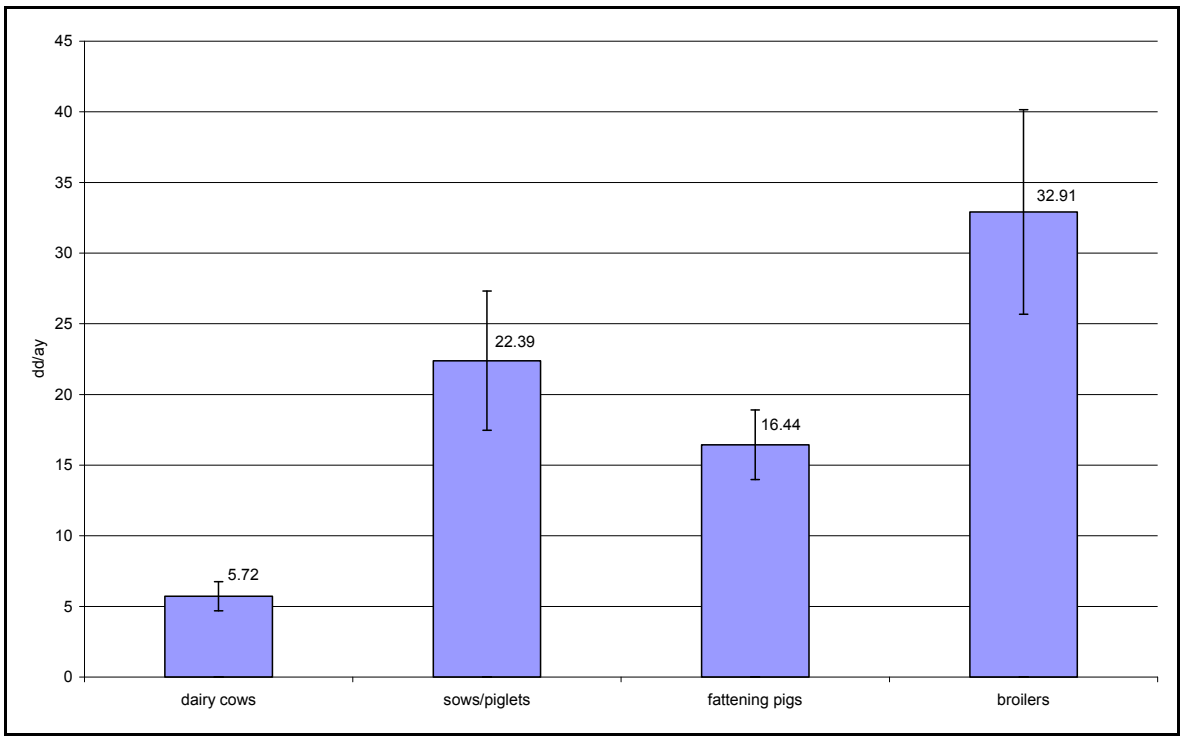
- A distinction can be made between three general treatment strategies in Europe
  - Scandinavian countries: a strategy primarily based on beta-lactam antibiotics
  - Denmark: a strategy based on tetracyclines + macrolides + betalactams
  - Other European countries: a strategy primarily based on tetracyclines
- Norway uses relatively large amounts of fluoroquinolones (15%) in the fish-breeding sector, which is more than the amount administered to humans in the Netherlands**.**
- All countries use roughly the same amount of trim /sulfa combinations (approx. 8%).

The above comparison has been made for all years from 2001 to 2006 inclusive, but holds for the years 1999, 2000 and probably 2007, as well.

3.3 Usage in the Netherlands in 2007

The information from the sentinel farms can be used to estimate the average antibiotic use in daily dosages per animal year per sector in the Netherlands**.** This figure indicates the daily dosages the average dairy cow, pig or broiler is administered per year. The average use, accompanied by 95% confidence intervals, is shown in Figure 3.8.

Figure 3.8 Average number of daily dosages per animal year in 2007, with 95% confidence intervals (total group)



These intervals indicate that with 95% certainty, the average antibiotic use in each sector in the Netherlands, expressed in terms of the number of daily dosages per animal year, will lie within the upper and lower limits indicated by the four vertical lines shown in Figure 3.8. The actual average use in the sectors in the Netherlands will be at most 15% (fattening pigs) to 22% (sows/piglets and broilers) higher or lower than the average determined at the sentinel farms**.**

3.4 Trends in antibiotic usage in the Netherlands

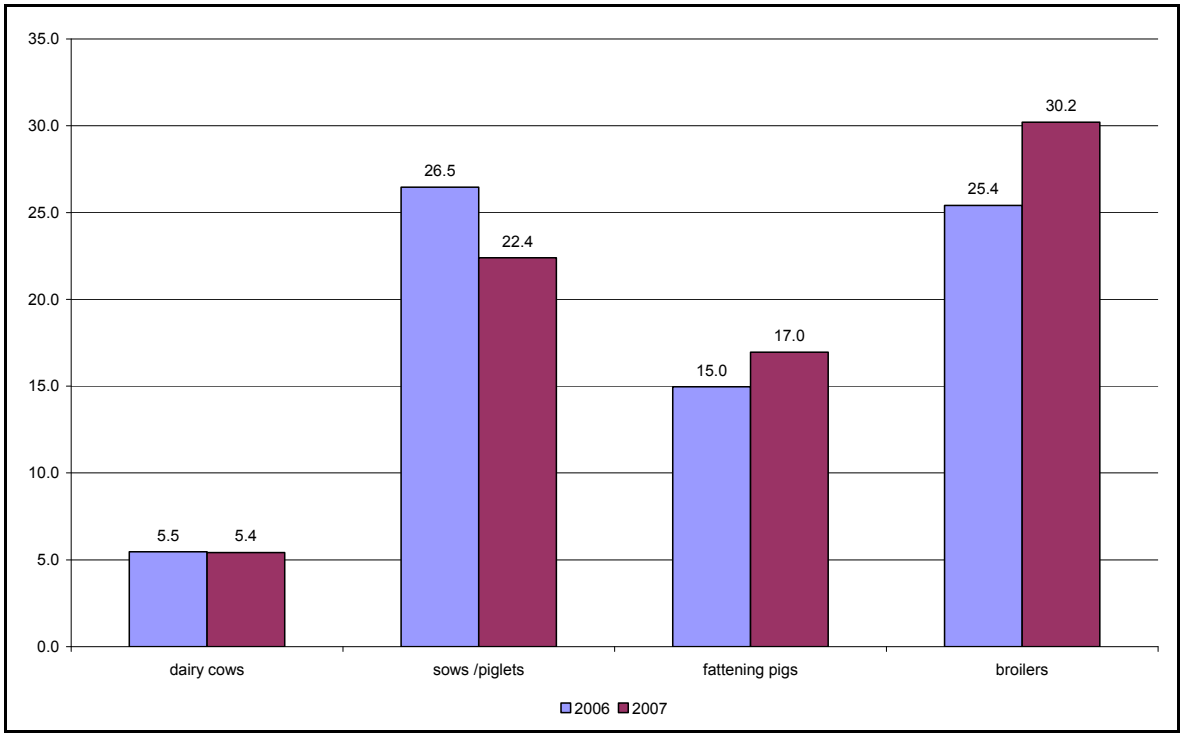
An insight into the variation in antibiotic use in the years 2006 and 2007 was obtained by analysing the figures of the 123 farms which took part in both years, i.e. the *comparison group*. Table 3.2 lists the figures for this group of farms as compared to the farms in the *total sample* for 2007.

Table 3.2 Differences in the average number of daily dosages per animal year between the comparison group and the total group.

		Dairy cattle	Sows/piglets	Fattening pigs	Broilers	Total
Number of farms	2007 total group	36	42	52	29	159
	06-07 comparison group	35	30	31	27	123
Number of daily dosages	2007 total group	5.7	22.4	16.4	32.9	n/a
	06-07 comparison group	5.4	22.4	17.0	30.2	n/a

Figure 3.9 shows the antibiotic use in the four sectors examined in this study expressed as daily dosages per animal year per average animal present. This figure reveals a tendency to increase in the antibiotic use in daily dosages administered to fattening pigs (+13.3%) and broilers (+18.9%). The daily dosages administered to sows/piglets decreased (-15.4%) and administered to dairy cattle remained virtually unchanged (-0.7%).

Figure 3.9 Trends in antibiotic usage 2006-2007 in daily dosages per animal year at farms in the comparison group 2006 – 2007



The number of farms in the *comparison group* differs from the *total group*, and this is the reason for the differences between figures 3.8 (and 3.10) and 3.9. The number of pig farms followed in 2007 increased substantially as compared to 2006 (+33 farms). However, notwithstanding the

difference in the number of farms, the difference in the average number of daily dosages per animal year between these groups is small. See Table 3.2.

This data does not permit a conclusion that the use in specific sectors in the Netherlands has increased or declined in consecutive years**.** None of the differences between consecutive years were significant. This is primarily due to the observed differences in use between the farms (large variation) in combination with a somewhat too-small number of farms in the sample

Figure 3.10 shows the trends in all farms in the sentinel farms from 2004 to 2007. If this study had been restricted to farms that have taken part in each of these years then the figures of only 77 farms would have been used. See also Annex 3.

Figure 3.10 Trends in antibiotic usage from 2004 to 2007 inclusive, expressed as daily dosages per animal year (total group)

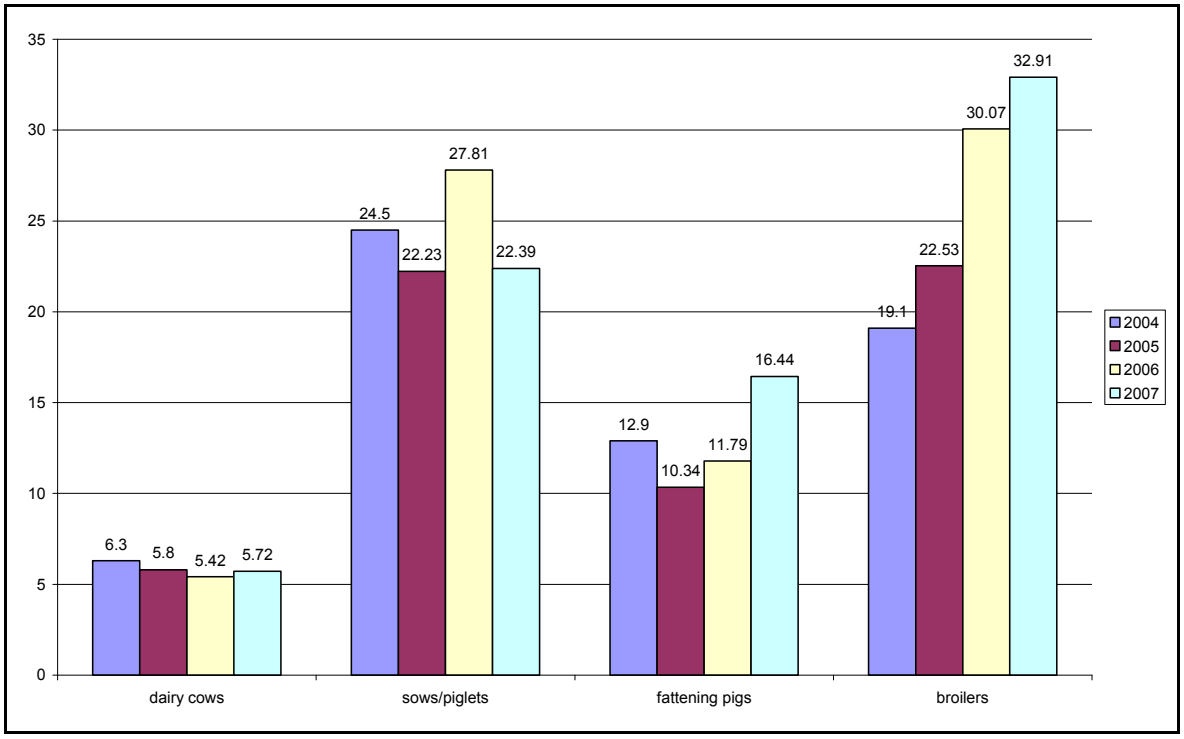


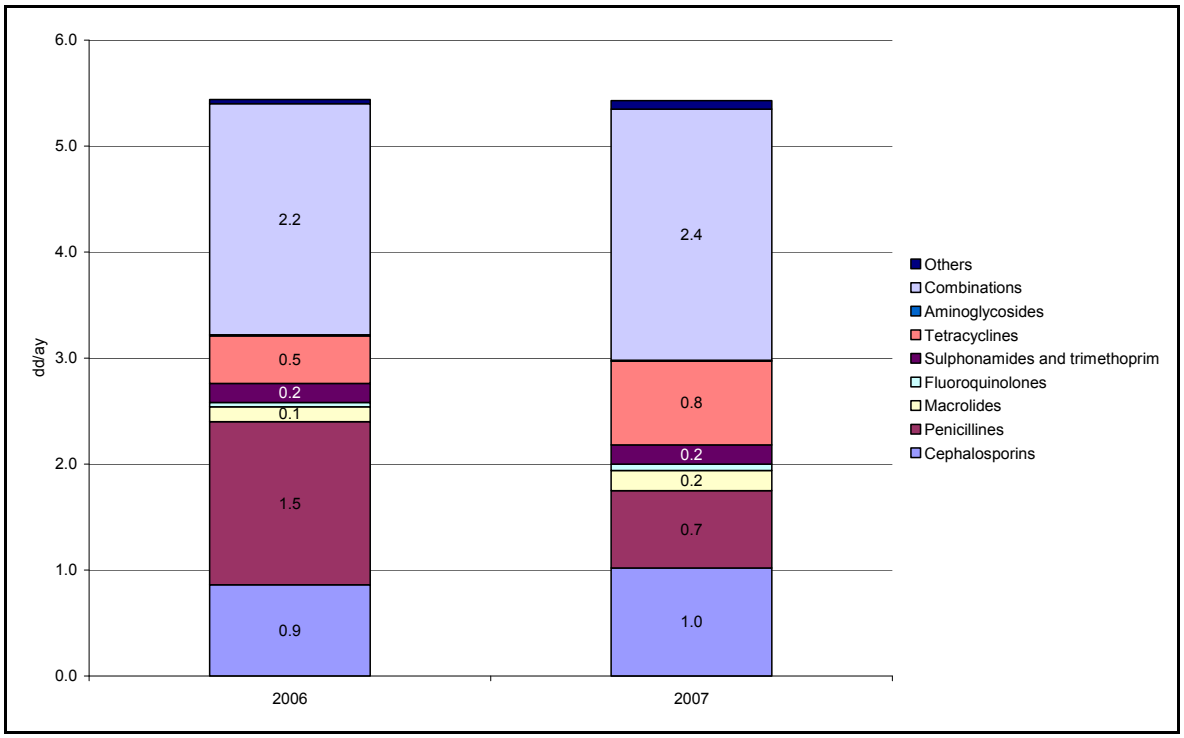
Figure 3.10 shows that the daily dosages administered at dairy farms are fairly constant; this is also the case at sow/piglet farms**.** The fattening pig and broiler farms exhibit increasing use; in both groups the use in 2007 is statistically significantly higher than the use in 2004.

The background data with details about the use in daily dosages per administration method is included in annexes 1a, 1b, 1c and 1d, and the background data with details about the use in grams of active ingredients is included in annexes 2a to 2d.

4. Dairy cattle

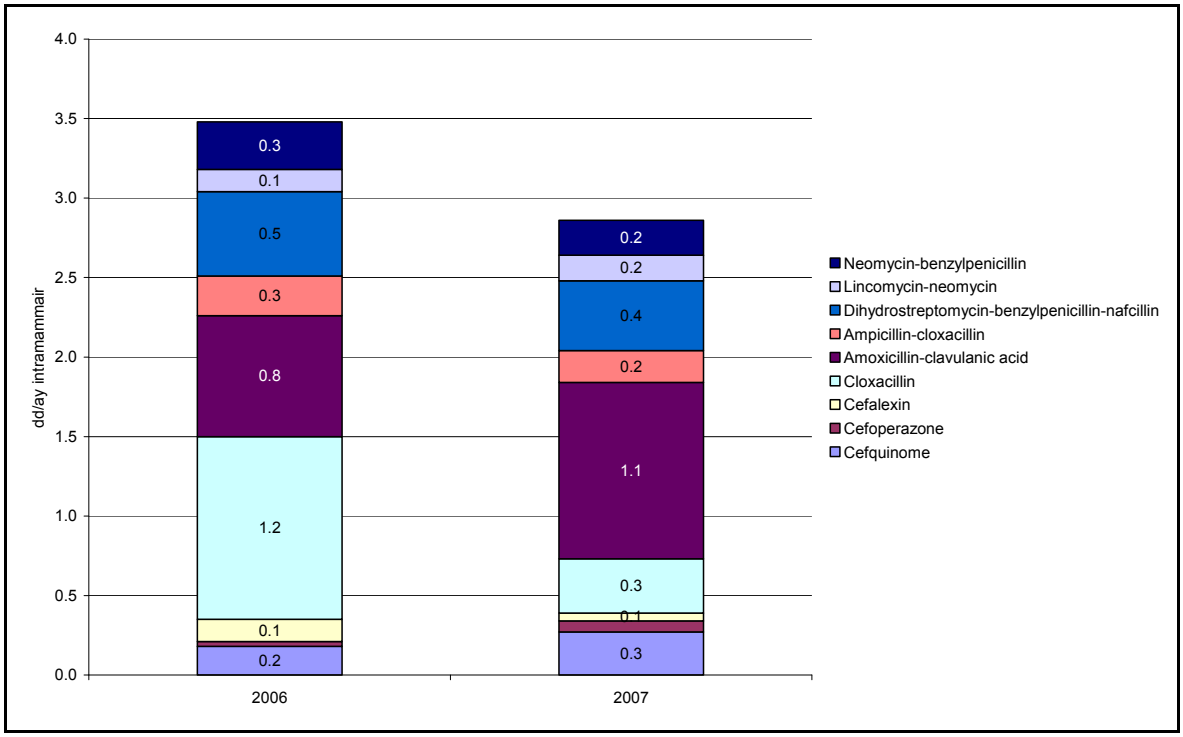
Antibiotic use remained virtually unchanged at the dairy farms**.** However, there were some shifts in the use of the various groups of antibiotics**.** The farms have used slightly more tetracyclines and less penicillins**.** See Figure 4.1.

Figure 4.1 Antibiotic use on dairy cattle in daily dosages per animal year (comparison group)



The percentage of the total antibiotic use for intramammary administration decreased from 64% in 2006 to 53% in 2007. Oral and parenteral administration has increased. The oral administration of doxycycline in particular, has increased (0.03 – 0.33). Figure 4.2 shows the various groups used for intramammary administration of antibiotics to dairy cows in 2006 and 2007. This reveals that there was a substantial increase in the use of amoxicillin clavulanic acid in 2007 as compared to 2006, as well as an increase in the use of cefquinome. The use of cloxacillin showed a substantial decrease.

Figure 4.2 Intramammary antibiotic use at active ingredient level on dairy cattle [dd/ay] (comparison group)

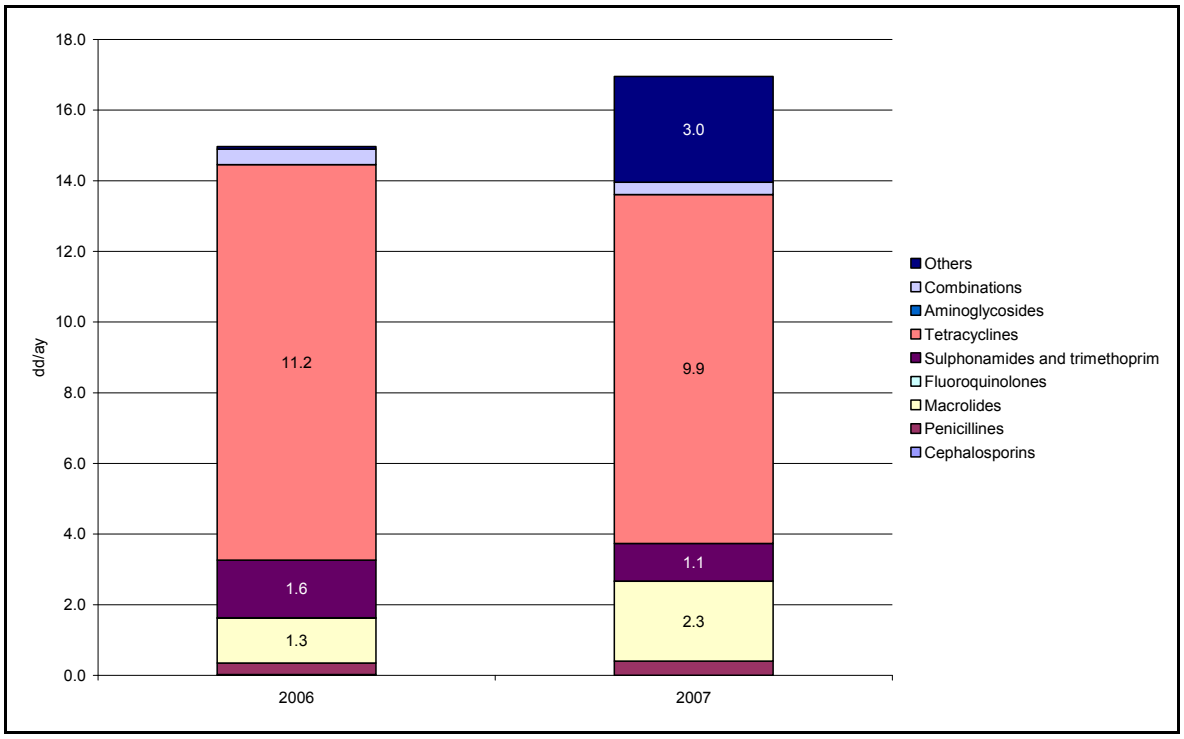


A relationship exists between the antibiotic use in 2006 and that in 2007 ($p = 0.00\ldots$; correlation coefficient = 0.678) and that in general a farm's antibiotic use in one year is a predictor of the antibiotic use in a following year. With other words farms with a high use in one year will often have a high use in the next year, whilst the use at farms with a low use in one year will often remain low.

5. Fattening pigs

The number of daily dosages in the comparison group 2006-2007 in this sample increased at the fattening pig farms from 15.0 to 17.0 (+13%). This increase is not statistically significant. Therefore it is not possible to conclude that there is an increase in antibiotic use at a national level on the basis of this data. See Figure 5.1.

Figure 5.1 Antibiotic use on fattening pigs by active ingredient, in daily dosage per animal year (comparison group)



Shifts have taken place in this sector. Tetracyclines are most commonly administered to fattening pigs: it is striking to note that the use of tetracyclines has declined sharply, certainly in view of the percentage of the total use, from 76% to 59% - and this in comparison with the increase observed from 2005 to 2006, which actually grew from 72% to 78%[9]. The other antibiotics reveal an increase of 3 daily dosages, which is almost entirely due to the increase in the use of colistin sulphate that was virtually unused in 2006. See also Annex 1b. The use of macrolides also increased (+1), due to tylosin. The use of trim /sulfa combinations decreased slightly (-0.6).

An analysis was carried out to review whether farms with a larger number of fattening pigs have a higher antibiotic use: this can be due to a higher disease pressure, but also to the shorter amount of time available for the care and inspection of each animal. The analysis revealed that in general farms with a larger number of fattening pigs have a slightly higher antibiotic use (p= 0.04; correlation coefficient = 0.324). No correlation was found between the time devoted to the animals and the number of daily dosages. There was a correlation between the time devoted to each animal and the size of the farm (p= 0.00; correlation coefficient = -0.656). However, it is not clear whether the higher antibiotic use was due solely to the size of the farm, or was also due to the shorter amount of time devoted to each animal.

[9] The difference between this figure (78%) for 2006 and the aforementioned 76% for 2006 is due to the fact that different groups of farms were examined in the two instances**.** The comparisons review farms followed in the two years**.** Consequently, for the comparison of 2005 and 2006 the group of farms used for 2006 was slightly different from that used for the comparison of 2006 and 2007.

An analysis was carried out to review whether farms with modern stalls have a lower antibiotic use. With fattening pigs the converse was, on the basis of this sample, visible: farms with partially modernised stalls have a significantly lower antibiotic use (17 daily dosages) than farms with modern stalls (27 daily dosages)[10] ($p= 0.04$; $n=32$). This relationship is not influenced by farm size.

A study was carried out to examine whether fattening pig farms with a high antibiotic use in one year also had a high antibiotic use in the next year, and vice versa. Analysis revealed a strong relationship between the use in 2006 and the use in 2007 ($p = 0.00$; correlation coefficient = 0.619). Consequently, it can be concluded that in general a fattening pig farm's antibiotic use in one year is a good predictor of the antibiotic use in a following year. This implies that farms with a high use in one year will often have a high use in the next year, whilst the use at farms with a low use in one year will often remain low.

The following variables did not yield any significantly different results for antibiotic use:

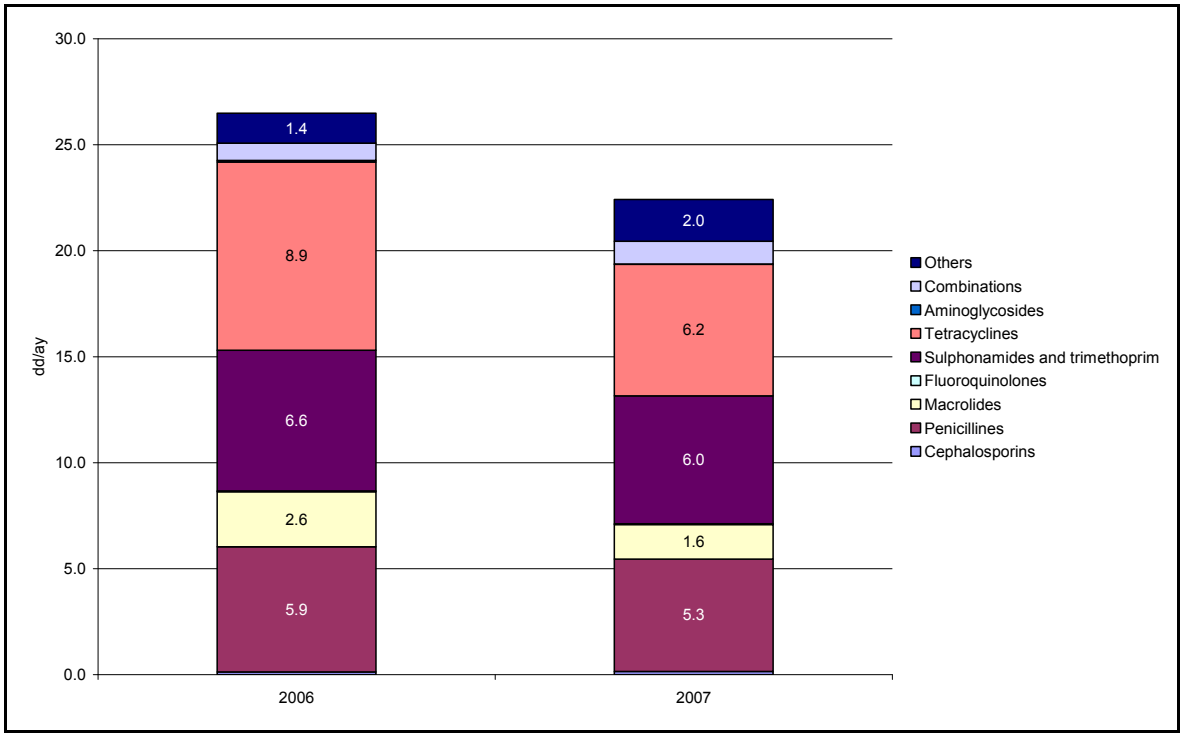
- Routinely, occasionally or never preventive treatments;
- The presence or absence of a farm treatment plan;
- The comprehensiveness of the farm treatment plan;
- The presence of employees;
- The use of basic feed;
- The fattening pig farmer's assessment of the health of the animals on his farm in 2007;
- The presence or absence of a standard preventive antibiotic treatment of piglets for fattening
- The extent to which slaughter anomalies are observed (impaired lungs/livers).

[10] There were too few farms with old or super-modern stalls, and for this reason they were excluded from the analysis.

6. Sows and piglets

The number of daily dosages decreased at the sow farms in the comparison group 2006-2007 in this sample from 26.5 to 22.5 (-15%). This is not a statistically significant decrease, and therefore it is not possible to conclude that there is a decrease in antibiotic use at a national level on the basis of this data. See Figure 6.1.

Figure 6.1 Antibiotic use on sows/piglets in daily dosages per animal year (comparison group)

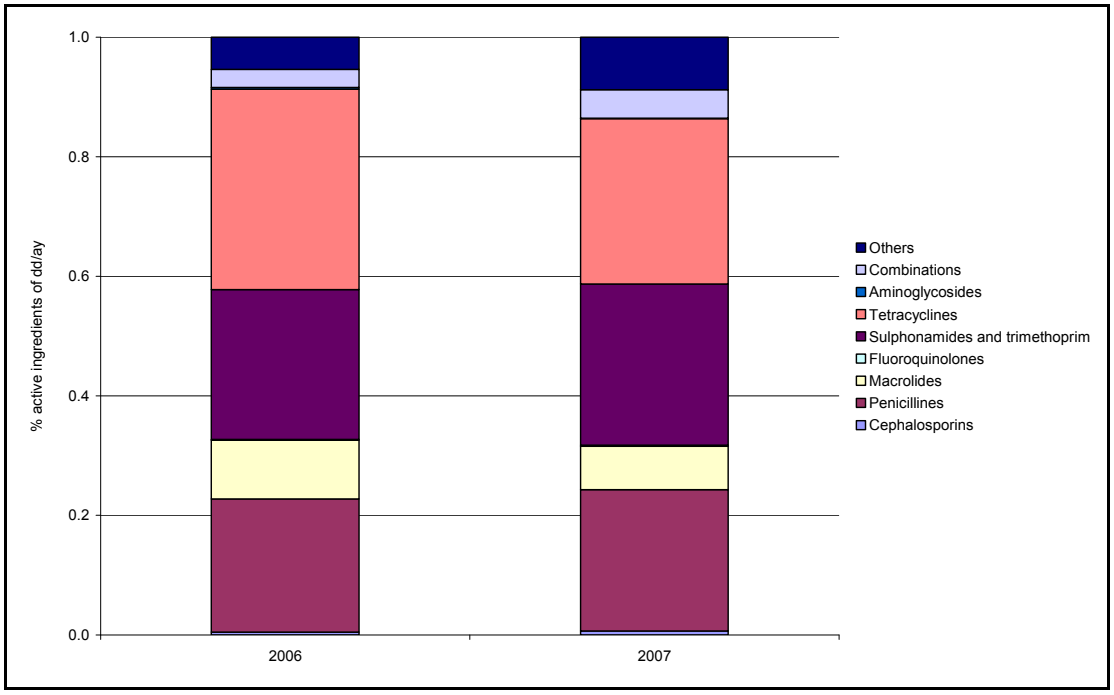


The decrease in the number of daily dosages was primarily due to the reduced use of tetracycline (–2.7 dd/ay). The use of most other antibiotic groups decreased to an extent such that the decrease in the proportion of the group in the total use was the same as the decrease in the total use. This is shown in Figure 6.2. Only the groups 'other antibiotics'[11] and 'antibiotic combinations'[12] exhibited a slight increase.

[11] Lincomycine and colistin sulphate

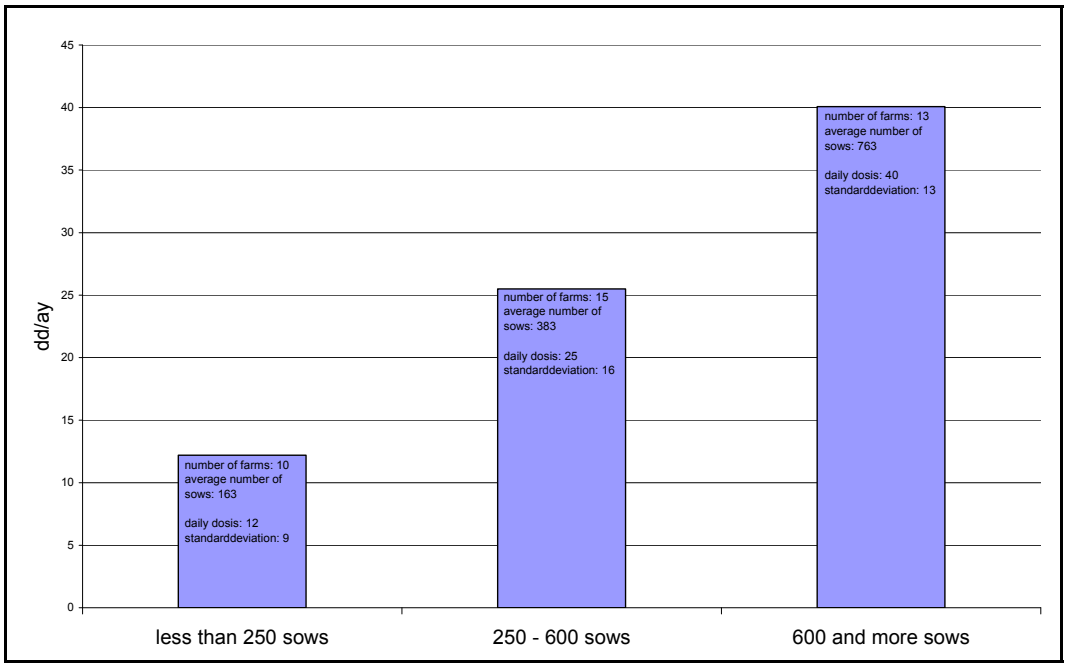
[12] Dihydrostreptomycin-benzylpenicillin, Lincomycin-spectinomycin and Neomycin-benzylpenicillin. See Annex 1c for the exact figures.

Figure 6.2 Antibiotic use on sows/piglets according to active ingredient, in percentage of daily dosages per animal year (comparison group)



An analysis was carried out to review whether farms with a larger number of sows have a higher antibiotic use: this can be due to a higher disease incidence, but also to the shorter amount of time available for the care and inspection of each animal. The analysis showed that in general farms with a larger number of sows have a higher antibiotic use ($p= 0.00$; $r^2 = 0.35$). Figure 6.3 shows that the antibiotic use at farms with 250 to 600 sows, is more than double the use at a farm with less than 250 sows. There is a statistically significant difference between the average numbers of daily dosages of all three groups in Figure 6.3.

Figure 6.3 Farm size (number of sows) related to the antibiotic use on sows/piglets (dd/ay)



The figures obtained from this sample reveal that farms with more sows have a higher antibiotic use per animal year. In addition, the figures also reveal that the time devoted to each sow decreases with the number of sows**.** However, there is no correlation between the spending of time and the antibiotic use . It would appear that there is another cause for the higher use of antibiotics at larger farms**.**

A difference has been found between the groups of sow farms with and without employees**.** Farms with employees have a higher antibiotic use (34 daily dosages per animal year) than farms without employees (18 dd/ay; p=0.00; sed = 4.6). The farmers were also asked whether the employees had completed a relevant course or had sufficient work experience. Only three farmers replied in the negative to this question.

It is expected that sow farms with different antibiotic policies will also exhibit differences in the quantities of antibiotics they use. Farms that occasionally made preventive use of antibiotics have the lowest use (15.8 dd/ay), farms that routinely employ preventative treatments have the highest use (33.6 dd/ay) and farms that never give preventive treatments lie in between the two (22.8 dd/ay). The highest and the lowest are statistically significant different (p = 0.00; sed = 4.1). This could be due to the fact that farms which occasionally make preventive use of antibiotics do so very deliberately on the recommendation of their veterinarian in response to a (past) problem. It is conceivable that farms which never use preventive treatments often respond to problems just too late and then need to introduce curative treatments that involve large amounts of antibiotics**.**

It is expected that sow farmers are able to make an accurate assessment of the health of their animals and that the use of antibiotics is a good indicator for this health status**.** Farmers stating that the health of their animals is poor do indeed have a high antibiotic use and vice versa (p=0.00; correlation coefficient = 0.474). The average daily dosage at the group with animals in moderate health was 36.7, while the average daily dose at the group who assessed the health of their animals as good was 20.6. None of the sow farmers assessed the health of their animals as poor.

Further analyses showed that sow farms which had a high antibiotic use in 2006 also had a high antibiotic use in 2007, and vice versa (p = 0.00; correlation coefficient = 0.716). Consequently, it can be concluded that in general a sow farm's antibiotic use in one year is a good predictor of the antibiotic use in a following year. This implies that farms with a high use in one year will often have a high use in the next year, whilst the use at farms with a low use in one year will often remain low.

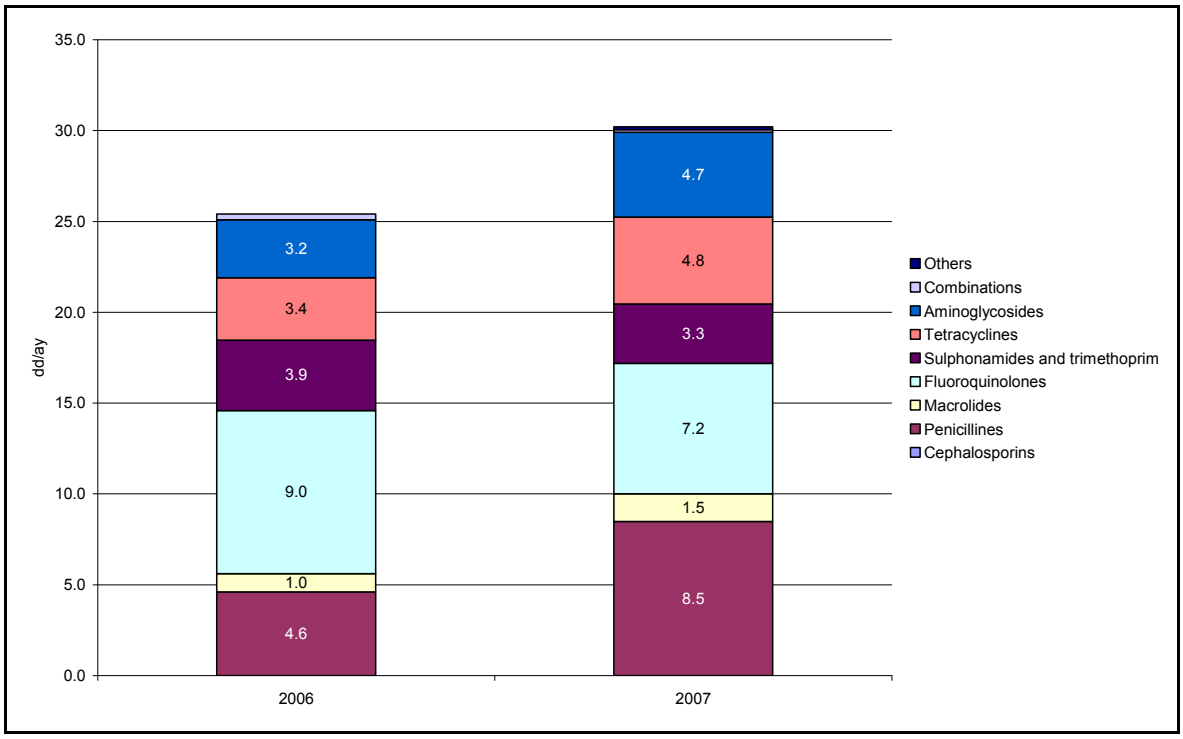
The following analyses did not reveal any relationships with the antibiotic use:

- The presence or absence of a farm treatment plan;
- The comprehensiveness of the farm treatment plan;
- The modernity of the stalls;
- The use of basic feed;
- The destination of the piglets (own farm, steady customers, other customers), and
- The number of piglets born live per sow.

7. Broilers

The number of daily dosages at the broiler farms in the comparison group 2006-2007 in this sample increased by 4.8: from 25.4 to 30.2 daily dosages (+19%). This increase is not statistically significant, and therefore it is not possible to conclude that there is an increase in antibiotic use at a national level on the basis of this data. See Figure 7.1.

Figure 7.1 Antibiotic use on broilers in daily dosages per animal year (comparison group)



The increase is largely due to the increase in the use of penicillins (+3.9) and, to a lesser extent, to the increase in the use of aminoglycosides (+1.5), tetracyclines (+1.4) and macrolides (+0.5). The use of fluoroquinolones (-1.8) and trim /sulfa combinations (-0.6) declined. The comparison of 2006 against 2005 had revealed an increase in the use of fluoroquinolones**.** The changes in the proportions of the various groups of antibiotics reflect the increases and decreases mentioned before. The proportions of the other groups were relatively unchanged.

In addition, it was remarkable to note that of the sample farms we analysed the broiler farms, in particular, made use of fluoroquinolones and aminoglycosides**.**

There are major differences between the antibiotic uses at the various farms: The 25% farms with the lowest antibiotic use administer an average of 8.7 daily dosages, the middle group (50%) administers an average of 30.9 daily dosages, and the major users (25%) administer an average of 67.8 daily dosages per animal year. This last 25% of the farms account for 49% of the total antibiotic use.

An analysis was carried out to examine whether farms using basic feed also had a different level of antibiotic use. The analysis showed that the antibiotic use at farms using basic feed was lower than farms that did not use basic feed ($p=0.05$; correlation coefficient = 0.371). The farms that did not use basic feed used feed with, for example, omega fatty acids, and a higher energy content. GMO-free feed, or feed in which the farmer could vary the percentage of wheat. An explanation for this could be that farms which supply special feed do so because of health problems**.**

An analysis was performed to examine whether there is a relationship between the size of broiler farms and antibiotic use. However, this was not the case.

An analysis was also performed to examine the relationship between the broiler farmer's assessment of the health of the animals and antibiotic use. The average number of daily dosages per

animal year at the farms of broiler farmers who assessed the health of their animals as good was 24; the average number of daily dosages per animal year at the farms of broiler farmers who assessed the health of their animals as moderate was 37; the average number of daily dosages per animal year at the farms of broiler farmers who assessed the health of their animals as poor was 51. However, as a result of the large dispersion in use and the small number of farms in the sample (n=27) the differences were not significant.

An analysis was performed to examine whether broiler farms with a high antibiotic use in one year also had a high antibiotic use in the next year, and vice versa. Further analysis showed a correlation between the use in 2006 and the use in 2007 ($p = 0.01$; correlation coefficient = 0.50). Consequently, it can be concluded that in general a broiler farm's antibiotic use in one year is a good predictor of the antibiotic use in a following year. This implies that farms with a high use in one year will often have a high use in the next year, whilst the use at farms with a low use in one year will often remain low.

The antibiotic use, expressed in daily dosages, is directly correlated with the health costs per broiler ($p = 0.01$; correlation coefficient = 0.467). As such this is not a surprise, since antibiotics account for 29% of the animal health costs of broilers (Bondt *et al.*, 2007).
The following analyses did not reveal any relationships with the antibiotic use:

The size of the farm;
Employees at the farm (yes/no);
The modernity of the poultry houses;
The use of preventive treatment (routinely, occasionally, never);
The poultry farmer's assessment of the health of the animals;
The number of bacteriological flock problems;
The number of problems other than bacteriological flock problems**.**

8. Discussion

Reliability of the results and sample size
The results in this study are based on data from sample farms in the Farm Accountancy Data Network. These results can be used to calculate the average use per average animal present on an average farm. The average value for the different sectors in the Netherlands has been calculated with a 95% confidence interval, i.e. on the basis of this sample it can be stated with 95% reliability that the average value for the Netherlands will be between certain lower and upper limits[13].

The number of farms in the sample depends on the required degree of certainty for the demonstration of possible differences in the use (increased or decreased use) in different years**.** The size of the sample is determined by establishing which increase or decrease in antibiotic use is deemed relevant and consequently needs to be detected as a statistically significant change. Table 8.1 lists the number of farms in the various sectors required to establish a difference in antibiotic use of 10, 15 and 20% with a 95% confidence interval ($\alpha = 0.05$). This is based on the dispersion currently exhibited by the sample. The dispersion exerts a great influence on the number of farms required in the sample: it should be noted that comparison group of farms and therefore also the dispersion in the figures used for the comparison of 2005 and 2006 differs from the comparison group and dispersion for the comparison of 2006 and 2007. Rows in which 'smaller than' is stated alongside a given figure indicate that the current sample size is already sufficient to demonstrate the difference. For example 'smaller than 31' stated in the fattening pig column (for a difference of 15%) indicates that the current study would have established that a 15% increase or decrease in antibiotic use was significant. The figures for comparing 2007 with 2006 revealed an increase of 13% (from 15 to 17 daily dosages, Figure 3.9): however, this difference is just short of significant. A sample of 49 farms would have been required to demonstrate a difference of 10% in fattening pig antibiotic use, based on the dispersion exhibited by the comparison group for 2006/2007.

[13] The confidence intervals were minimised by using data from the Agricultural Census, whereby the estimate takes into account the farm size using weighting factors.

Table 8.1 Optimum sample size for the demonstration of the specified % differences in antibiotic use between different years (α =5%)

% difference	Basic S[14]	Dairy cattle	Sows	Fattening pigs	Broilers
10%	05-06	46	43	89	84
	06-07	62	105	49	63
15%	05-06	smaller than 34	31	59	57
	06-07	44	60	smaller than 31	42
20%	05-06	smaller than 34	smaller than 27	44	45
	06-07	smaller than 35	39	smaller than 31	33

Table 8.1 shows that the current sample is too small to demonstrate a 10% increase or decrease of the use of antibiotics**.** For that reason the monitoring for 2008 provides for a substantial expansion of the number of farms in the dairy and pig sectors. This expansion is also desirable in the broiler sector. A larger sample size will improve the accuracy of the estimation of the actual national use and, in so doing, lay firmer foundations for further studies into the background reasons for the major differences in antibiotic use revealed by the data. Supplementary information could be obtained by organising a workshop with participants from the sample: it is expected that this will provide an improved insight into the reasons for the major differences in use.

It is not currently possible to employ the usage figures from the monitoring in the Data Network to calculate antibiotic use at a national level, primarily because veal farms have yet to be included in the sample. The veal sector has now begun to implement the Rational Antibiotic Use Master Plan, and within this context antibiotic use is being monitored from 2007 onwards**.** It is expected that the first results will be announced in the annual MARAN report published at the beginning of 2010. The availability of this data should provide an opportunity for a comparison with the (national) FIDIN data.

One of the current characteristics of the method used to calculate the daily dosages is the use of average animal weights**.** This assumes that the probability that an animal will be treated with antibiotics is independent of the age of the animal. However, this is not the case: younger animals have more frequent health problems than older animals, while animals no longer receive antibiotics in the last period before slaughter to ensure that the meat is free of antibiotic residues**.** An improved estimation of the treatment duration would be obtained by replacing the average weight expressed in terms of the average weight during the animals' presence on the farm by the best possible estimate of the average weight *on treatment*. This could possibly be achieved by collecting usage figures at herd level and assuming that the date of purchase of a preparation is also the date of treatment. The age (and related weight) of the animals treated could be estimated at this treatment date. This more accurate method of calculation is already being used in the monitoring of antibiotic use in the veal sector. Therefore we emphasize that restraint is needed in comparing the use of antibiotics (number of dd/ay) between different sectors**.**

On closed farms, the weights of the animal present are calculated on the basis of the factors including the numbers of fattening pigs and piglets and a standard weight per animal. The accuracy of this estimation can be improved if information is available about the weights at which piglets on individual farms move on to fattening. The weaned piglets, in particular, are treated at sow farms: once again, the appropriate designation of the preparation to the animal group and age/weight would provide an improved view into the antibiotic use and actual differences between farms**.**

[14] S indicates the estimate of the required sample size (σ). This column specifies the data set used to estimate σ.

Comparison of countries
Compared to other countries of which figures of veterinary antibiotic use are available, the use of antibiotics per animal in the Netherlands is high. It is not clear whether or not this higher usage is caused by specific sectors or that the usage is higher in all sectors of animal production. Unfortunately, the figures of the other countries can't be specified into use per animal production sector.

Use of antibiotics: a matter of health but also of management
There are big differences in the usage of antibiotics between farms**.** At broiler farms for example the 25% major users account for 49% of the total antibiotic use.

The analysis of the figures of all animal species shows that there is a positive correlation between the use of antibiotics in one year and the previous year. This could mean that the animal health situation at those farms is structurally bad, or it could be caused by the management of the farmer. Indications for the impact of animal health management mainly emerge at the sow farms**.** Further analyses at those sow farms indicates the following:

a) farms with more sows generally have a higher use of antibiotics; however, the variation in use between farms also increases with increasing farm size;
b) farms that occasionally used antibiotics preventively on average have the lowest use, farms that routinely employ preventative treatments have the highest use and farms that never give preventive treatments lie in between the two; When the decision to use preventive use antibiotics preventively is a deliberate choice based on medical grounds, this appears to result in less total antibiotic use at these farms;
c) the assessment of sow farmers on the health of their animals is consistent with their level of antibiotic usage; the better the estimated health status of the farm, the lower the usage of antibiotics**.**

9. Conclusions

The results from the monitoring give a broad and detailed view of the usage of various antibiotics at dairy, pig and broiler farms and the trends in their use.

The analysis of the FIDIN figures shows that the total therapeutic veterinary antibiotic use including antimicrobial growth promoters (AMGPs) increased by over 3% in the years from 1999 to 2007. During this same period, the growth promoters have been banned, first partly and as from 2006 entirely and the therapeutic veterinary antibiotic use increased by 83%, an average increase of 7.9% per annum. The therapeutic usage increased by 8.9% in 2007 as compared to 2006. The therapeutic antibiotic use per kg live weight in 2007 was twice that in 1999. Part of this increase may be accounted for by a substitution of growth promoters**.**

In comparison with other countries for which veterinary antibiotic use figures are available the antibiotic usage per animal is highest in the Netherlands**.** However, it is not yet clear whether this is applicable to all or only some sectors**.** Antibiotic usage is increasing in the Netherlands, Denmark and Germany, while the use is stable in five other countries examined in this study.

The antibiotic usage in 2007 at the 159 sentinel farms from the Farm Accountancy Data Network is as follows: dairy cattle 5.7 daily dosages per animal year ($\pm$ 1.0[15]); sows/piglets 22.4 daily dosages per animal year ($\pm$ 5.0); fattening pigs 16.4 daily dosages per animal year ($\pm$ 2.5); broilers 32.9 daily dosages per animal year ($\pm$ 7.3).

The figures on the antibiotic usage in the Netherlands expressed in terms of daily dosages per animal as based on all farms from the samples for 2004 to 2007 inclusive reveal that antibiotic usage has remained fairly stable for dairy cattle and sows/piglets**.** The antibiotic usage in fattening pigs and, broilers in particular, shows a clear increase. The increase in fattening pigs is statistically significant and numbers 3.5 daily dosages per animal year (95% confidence interval: 2.0 to 11.5 daily dosages per animal year). The increase in broilers is also statistically significant and numbers 13.8 daily dosages

[15] The figures between brackets indicate the bandwidth. Consequently, the actual average antibiotic use per sector in the Netherlands will, with 95% confidence, lie between the specified upper and lower limits: for example, with dairy cattle between 4.7 and 6.7 daily dosages per animal year.

per animal year (95% confidence: 4.1 to 27.0 daily dosages per animal year). These confidence limits were calculated on unweighted data.

The antibiotic usage in *dairy cows* has remained relatively unchanged at 5.4 daily dosages per animal year. The proportion of antibiotics administered via the udder (intramammary) fell from 3.5 daily dosages per animal year in 2006 to 2.9 in 2007, whereby the use of cloxacillin decreased and the use of amoxicillin clavulanic acid increased.

Antibiotic usage in *fattening pig* increased from 15 to 17 daily dosages per animal year. The use of tetracyclines has decreased (–1.3 dd/ay) and the use of other antibiotics (colistin sulphate; +3 dd/ay) and macrolides (+1 dd/ay) increased.

The antibiotic usage in *sows/piglets* in 2007 was comparable to that in 2006, both in terms of the total use and the use of the various groups of antibiotics**.** The comparison of 2006 and 2007 shows a decrease from 26.5 to 22.4 daily dosages administered to sows/piglets per animal year. Further analysis of the sow farms revealed that: a) in general, antibiotic use is higher at farms with more sows; b) farms that occasionally use antibiotics preventively have the lowest average antibiotic use, while farms that routinely give preventive treatments have the highest average use and the use at farms that never give preventive treatments lies in between the two; c) the sow holders' assessment of the health of their animals is related to their use of antibiotics: antibiotic use decreased in proportion to the farmers' assessment of the appropriate health of their animals**.**

The total antibiotic usage in *broilers* increased considerably from 25.4 dd/ay in 2006 to 30.2 in 2007 (comparison group 2006/2007), mainly due to the increased use of penicillins (+3.9 dd/ay; amoxicillin and phenoxymethylpenicillin). Fluoroquinolones and amino glycosides are primarily used in the broiler sector, although the use of fluoroquinolones decreased (-1.8 dd/ay). Antibiotic use varies greatly between farms: for example 25% of the broiler farms account for 49% of the total antibiotic usage.

Farms in *all sectors* that used a large amount of antibiotics in one year mostly also used large amounts of antibiotics in the previous year. Consequently, antibiotic use at farms is relatively stable.

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II Resistance data

In this chapter susceptibility test results are presented as determined in 2006/2007 for the food-borne pathogens *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* O157, the food-borne commensal organisms *E. coli*, *Enterococcus faecium* and *E. faecalis*.

Food-borne pathogens

Salmonella

In this chapter resistance percentages are presented on salmonella's isolated from humans with clinical infections, food-animals and their products, as potential sources for distribution to humans via the food chain, and animal feeds as potential source for food-animals and their products.

Highlights

In 2006/2007 *S.* Typhimurium and *S.* Enteritidis were the most prevalent serovars in humans, third was the antigenic variant *S. enterica* subspecies *enterica* 1,4,5,12:i:-, a so called monophasic variant of *S.* Typhimurium which is emerging internationally since 2004. Pigs and cattle were the most important animal sources of *S.* Typhimurium. Layers (eggs) and foreign travel were the most important sources for *S.* Enteritidis. In broilers *S.* Java was isolated most frequently. In broilers *S.* Enteritidis and *S.* Typhimurium constitute only a small fraction of all salmonella's.

In 2006/2007 81 cefotaxime resistant, ESBL suspected strains were found, which was substantially more than in 2004/2005 (n = 21). These isolates belonged predominantly to the serovar *S.* Java (63%) of which only 1 strain was isolated from a human source, the others all from poultry sources. Resistance to cefotaxime is increasing at an alarming rate. The isolates from humans originate partly from exotic sources, but also from poultry. In animals poultry is up to now the only reservoir for ESBL-producing salmonella's.

A total of 16.861 Salmonella isolates were tested for antimicrobial susceptibility between 1999-2007. Human isolates (N=9155) concerned a selection from first isolates sent to the Dutch National Institute of Public Health (RIVM) by the regional public health laboratories. All strains were the first isolates recovered from patients with salmonellosis. The majority of the isolates from pigs (N=1124) and cattle, including calves (N=600) were sent to the RIVM by the Animal Health Service from a diversity of surveillance programs and clinical Salmonella infections. Those from chickens (broilers, including poultry products, N=1149; layers, reproduction animals and eggs, N=827) concerned mainly nonclinical Salmonella infections derived from a diversity of monitoring programs on the farm, slaughterhouses and at retail. The majority of isolates from layers in 2005 concerned those from the Dutch component of the EU-baseline study. Isolates from a diversity of other sources have been analysed as well (animal feed and human food products; other animals from animal husbandry and pets, samples from the environment, etc.).

In 2006/2007, *S.* Typhimurium and *S.* Enteritidis were the most prevalent serovars isolated from humans in The Netherlands (table 9). Third was the antigenic variant *S. enterica subspecies enterica* 1,4,5,12:i:-, a so called monophasic variant of *S.* Typhimurium which is emerging internationally since 2004 (Mossong J, et al. Outbreaks of monophasic *Salmonella enterica* serovar 4,[5],12:i:- in Luxembourg, 2006. Euro Surveill 2007;12(6))

Like in 2004/2005 in pigs *S.* Typhimurium was by far the most prevalent serovar and in cattle *S.* Dublin. In poultry a difference existed in prevalence of serovars between broilers and layers. In isolates from broilers *S.* Paratyphi B var. Java (*S.* Java) and *S.* Infantis predominated and in layers *S.* Enteritidis and *S.* Senftenberg.

Travel contributed from 0% to almost 40% of the cases of human salmonellosis depending on the sero/phagetype. Of the two most frequently isolated human serovars, travel contributed substantially more to the incidence of *S.* Enteritidis than *S.* Typhimurium. Travel contributed to 46% of the *S.*

Kentucky cases in humans, the serovar most commonly harbouring a high level of resistance to ciprofloxacin. Travel is, however, strongly underreported, by about a factor of two.

Table 9. Most prevalent Salmonella sero-, and phagetypes isolated in 2004/2005 and 2006/2007 from humans, pigs, poultry, broilers and layers16 and the % travel related infections in 2004 – 2005.

		Humans		Pigs		Cattle		Poultry		Broilers		Layers	
		04/05	06/07	04/05	06/07	04/05	06/07	04/05	06/07	04/05	06/07	04/05	06/07
		N	N	N	N	N	N	N	N	N	N	N	N
Total number sent to RIVM		3384	3320	599	581	284	312	1204	1034	549	464	457	163
Sero/phagetype	Travel	% of the total sent to RIVM											
Typhimurium	4%	33.9	33.7	54.8	44.2	14.8	26	6.5	4.8	6.2	5.4	6.1	6.7
DT104	3%	11.8	4.8	16.5	9.6	4.6	4.5	2.7	1.7	2.2	1.7	3.1	2.5
ft507	2%	6.5	8	14.5	9.3	4.2	8.7	1.2	1	2	1.3	0.4	
ft510	8%	1.4	1.3	1.2	2.4	0.7	0.6						
ft508	2%	1.2	0.3	1.7	0.7			0.3		0.5		0.2	
ft561 (DT7)	0%		7.2		1.2		2.9						
ft296	4%		1		0.2		0.3		0.1		0.2		
Enteritidis	13%	40.2	35.5		3.1	0.4	2.9	17.5	6.8	6.9	3	33.3	19
Pt21	16%	7.2	4.7				0.3	2.6	0.9	3.1	0	3.1	4.9
Pt8	9%	4.3	2.7		0.5	0.4	0.6	1.7	0.4	0.5	0.2	2.4	0.6
Pt4	8%	12.3	14.3		0.5		1	6.3	2.9	1.6	0.9	12.9	6.7
Pt1	24%	4.2	3		0.2			1.1	0.8	0.5	0.9	1.5	0.6
Pt6	10%	3.7	3.8		0.2			1.2	0.1			2.8	0.6
Pt6a	26%	1.3	1		0.2			0.2	0.4			0.4	0.6
Pt14b	17%	1.2	0.8		0.2			0.3	0.1	0.2	0.2	0.7	
Pt3	22%	0.3	0.8										
Dublin	0%	0.4	0.5	0.2	0.2	53.5	56.4	0.2					
Paratyphi B var Java	5%	0.1	0.5	0.3	0.2			18.3	33.8	30.6	32.8	2	1.2
Infantis	15%	1.4	1	1.5	4.3	0.4	1	9.5	13.1	13.8	16.2	3.9	4.3
Senftenberg	24%	0.3	0.6	0.3	0.9			9.2	5.7	2.6	2.4	18.6	27
Mbandaka	23%	0.3	0.3	0.2	0.7	2.1	0.3	3	2.2	3.5	3.7	2	1.8
Virchow	40%	1.1	1.6		0.5	0.7		5.4	7	2.9	7.8	7	4.3
Derby	13%	0.8	0.5	12.9	10.5	1.4		1.1	0.3	1.6	0.6	0.7	
Livingstone	4%	0.3	0.2	2	2.8			1.6	1.6	1.3	2.4	2.2	1.2
S1,4,5,12:i:-	2%	0.9	4.7	0.8	9.1	1.4	5.8	0.2	1.1	0.4	1.9		
Brandenburg	3%	1.3	0.5	2.8	4	1.8		0.3		0.2		0.7	
Heidelberg	13%	0.7	0.5		0.2			0.2	2.4		1.1		5.5
Gallinarum	0%		0.1					0.7	2.2			1.8	13.5
Hadar	31%	0.9	0.8					1.5	2.5	2	2.8	0.7	
Indiana	10%	0.2	0.2					1.1	2	0.9	2.4	0.2	
Agona	26%	0.4	0.5	0.5	1.2			2.7	1.5	1.5	1.9	4.2	0.6
London	4%	0.6	0.2	0.8	3.4	0.4	0.3	0.6	0.7	1.1	1.3	0	0
Thompson	12%	0.1	0.2		0.5	0.7		0.2	1.1	0.2	0.4	0.4	4.9
Kentucky	36%	1.1	1.1			0.4		1.7	0.5	1.1	0.4	3.1	1.8
Anatum	25%	0.4	0.3	5.2	0.3	5.3		0.7	1.4	1.3	1.9		
Saintpaul	32%	0.8	0.6		0.2		0.3	0.3	2.2	0.5	2.8		0.6
Montevideo	17%	0.3	0.4		1	0.4	1.3	0.9	0.2	0.7		1.1	
Goldcoast	4%	0.5	0.4	0.3	1.4	1.4	1.6	0.2	0.1	0.2	0.2	0.2	
Panama	4%	0.5	0.4	3.2	1.4		0.6	0.2		0.2			0
Bovismorbificans	10%	0.4	0.3	0.7	1.4	0.4	0.3	0.2	0.3	0.5	0.2		1.2
Newport	21%	0.7	1.1	1	0.2	0.4		0.2		0.2			
Corvallis	28%	0.9	0.7					1.2	0.3	1.8			
Stanley	28%	0.5	0.5					0.1	0.3		0.4	0.2	
(Para)Typhi (A B C)	33%	2	1.9										
Other		8.1	10.2	12.5	8.4	14.4	3.2	14.4	6.1	17.9	8	11.8	6.1

Typing results of the Dutch Salmonella Reference Laboratory (RIVM, Bilthoven). Isolates are from different sources and programs**.** Poultry: all chicken categories together; Broilers: including chicken products; Layers: including reproduction animals and eggs**.**

[16] Source: Report on trends and sources of zoonotic agents in the EU, 2007, The Netherlands

Table 10. MIC distribution (in %) and resistance percentages (R%) for all salmonella's (N = 4642) tested **for antibiotic susceptibility in 2006/2007.**

Salmonella	MIC (%) distribution mg/L																	
N = 4642	0.015	0.03	0.06	0.13	0.3	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin						15.2	57.6	6	0.6		0.1	0.1	2.2	18.2					20.6
Cefotaxime			5.2	83.7	7.9	1.4	0.1		0.1	0.3	0.2	0.9							1.7
Ceftazidime				11.7	65.9	19	1.6	0.2	0.2	0.1	0.2	1							1.5
Gentamicin					31.8	56	10.1	0.6		0.4	0.5	0.3	0.3						1.5
Neomycin							89	8.5	0.6			0.2	0.5	0.5	0.7				1.9
Tetracycline						0.1	20.3	53.9	5.3	0.5	0.1	3.7	3.3	12.8					20
Sulphamethox.										43.7	34	0.7				0.1	0.1	21.4	21.6
Trimethoprim						84.8	1.9	0.2					2.1	11.1					13.2
Ciprofloxacin	0.5	8.5	77.7	2	6.2	2.9	1.5	0.3		0.1	0.1	0.2							13.3
Nalidixic acid								20	61.8	5.3	1	0.1	0.1	2.2	9.6				12
Chloramphenicol								0.1	4.4	72.8	15.7	0.4		1.2	5.4				7
Florfenicol								0.5	38.2	53	3.3	1.3	2.7	0.4	0.5				4.9
Streptomycin								8.2	20.1	21.9	23.4	10.2	4.1	3.9	8.2				16.2
Kanamycin									87.4	7.4	1.3	0.2	0.2		3.5				5.2
Colistin										100									0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. The vertical bars indicate the epidemiological cut-off values we used the calculate the resistance percentages, the dashed bars indicate clinical breakpoints**.**

Table 10 presents MIC-distributions and resistance percentages of all salmonella's tested for susceptibility in 2006/2007. Highest levels of resistance were observed for ampicillin, tetracycline, sulphamethoxazole, streptomycin and to a lesser extend chloramphenicol, ciprofloxacin, nalidixic acid and trimethoprim.
In 2006/2007 81 cefotaxime resistant, ESBL suspected strains were found, which was substantially more than in 2004/2005 (n = 21). The isolates belonged predominantly to the serovar *S.* Java (63%, N = 51) of which only 1 strain was isolated from a human source and the other 50 all from poultry sources. Other ESBL-suspected serovars were Agona, Braenderup, Concord, Cubana, Enteritidis Pt1, Pt4, Pt6a, Indiana, Infantis, Saintpaul, Senftenberg, Typhimurium Ft 508, Ft80 and Virchow. Of all these ESBL suspected salmonella's 19.7% (N = 16) were isolated from humans and the rest almost all from poultry sources. Thirty six of these isolates (44%) were resistant to nalidixic acid and also showed increased MICs for ciprofloxacin (MIC 0.25 – 2 mg/L). Four isolates (3 Concord and 1 Senftenberg) showed a phenotype typical for the presence of plasmid mediated quinolone resistance (MIC ciprofloxacin 0.5 – 1 mg/L and nalidixic acid 8 – 16 mg/L). The presence of qnr-genes was confirmed by pcr.
Resistance to cefotaxime is increasing at an alarming rate. The isolates from humans originate partly from exotic sources (eg. Africa), but also from poultry. In animals poultry is up to now the only reservoir for ESBL-producing salmonella's.

Using the epidemiological cut off value of 0.06 mg/L, 618 isolates were detected that demonstrated a non-wild type phenotype for ciprofloxacin. Of these, 32 (0.7%) sowed MICs larger that the clinical breakpoint (1 mg/L). The serovars of these ciprofloxacin resistant isolates were predominantly *S.* Kentucky (50%) and *S.* Java (25%). Since 2002 annually high-level ciprofloxacin resistant *S.* Kentucky's were isolated from human patients. These strains are related to travel to North African countries and genetically closely related because they all harbour a class 1 integron with gene cassettes aacC-A5 and aadA7 encoding for aminoglycoside resistance, moreover these isolates mostly harbour an incomplete Salmonella genomic island 1, failing the multi-drug resistance region resulting in the typical ACSSuT phenotype.

Table 11. Resistance (%) of the thirteen most prevalent Salmonella serovars isolated in The Netherlands in 2006/2007.

	Typhimurium (1053)	Enteritidis (1032)	Java (210)	Dublin (187)	Senftenberg (182)	Infantis (155)	Virchow (99)	Agona (82)	Mbandaka (78)	S. subsp. enterica 1.4,[5],12:i:- (76)	Derby (70)	Livingstone (69)	Hadar (66)
Ampicillin	45.8	5.2	58.6	1.6	0.5	20.6	36.4	11	1.3	82.9	10.0	5.8	33.3
Cefotaxime	0.2	0.5	24.3	0	0.5	5.2	3	1.2	0	0	0	0	0
Ceftazidime	0	0	21.4	0	0.5	3.9	3	1.2	0	0	0	0	0
Gentamicin	0	0	2.4	1.1	1.1	1.3	4	1.2	0	2.6	0	1.4	1.5
Neomycin	1.6	0	5.6	0.5	0.6	3.0	22.7	0	0	3.8	4.5	1.7	0
Tetracycline	48	1.6	24.3	2.1	1.1	6.5	28.3	4.9	1.3	82.9	31.4	2.9	89.4
Sulphamethox.	46.5	1.4	56.2	7	2.2	19.4	45.5	13.4	2.6	85.5	24.3	7.2	22.7
Trimethoprim	15.5	1	93.8	1.6	2.2	16.1	45.5	9.8	1.3	11.8	20	7.2	21.2
Ciprofloxacin	3.7	13.5	53.3	3.7	3.8	10.3	77.8	6.1	7.7	2.6	2.9	1.4	81.8
Nalidixic acid	2.8	12.9	47.1	3.2	2.7	9.0	77.8	4.9	6.4	2.6	2.9	1.4	81.8
Chloramphenicol	21.4	0	2.4	5.9	0.5	3.2	7.1	4.9	3.8	7.9	4.3	4.3	0
Florfenicol	19.3	0	0	0	0	1.3	0	1.2	1.3	0	2.9	0	0
Streptomycin	40.9	0	38.8	20	0	13.6	18.2	0	8.3	75.0	0	0	66.7
Kanamycin	6.1	1	10.2	0	0	0	9.1	0	8.3	8.3	0	0	33.3
Colistin	0	0	0	0	0	0	0	0	0	0	0	0	0

In table 11 resistance percentages are presented for the twelve most prevalent serovars isolated in The Netherlands in 2006/20075. The highest resistance levels are observed in *S.* Typhimurium, *S.* Java, the monophasic *S. enterica subspecies enterica* 1,4,[5],12:i:-, *S.* Hadar and *S.* Virchow.

S. Enteritidis

In table 12 resistance percentages for *S.* Enteritidis and it most prevalent phage types are presented. In The Netherlands, human infections caused by *S.* Enteritidis are predominantly related to the consumption of raw shell eggs**.** In Dutch broilers and broiler products the prevalence of *S.* Enteritidis is lower (Tables 9 and 15). The difference in phage type distribution and resistance profile of strains from human infections and Dutch poultry indicates that other sources of infection exist. In 2006/2007 from human infections, 122 ciprofloxacin non wild type susceptible strains were isolated, predominantly Pt1 (48%) and to a lesser extend Pt4 (16%). In Dutch layers in 2005 for the first time ciprofloxacin non-wild type strains were found (37%). Of the isolates 66% was Pt 4 and 2% Pt1. In 2006/2007 from Dutch layers only 2 ciprofloxacin reduced susceptible *S.* Enteritidis isolates were detected (Fig. 10).
The sudden high level of resistance in *S.* Enteritidis Pt4 from layers in 2005 was a very striking observation. Quinolone resistance is not likely to be selected through usage in layers because in these animals the use of fluoroquinolones or flumequine is not licensed. Therefore, the most likely explanation for this phenomenon was incidental introduction of quinolone resistant *S.* Enteritidis Pt4 by importation of contaminated eggs or breeding animals, which was repaired in 2006/2007.

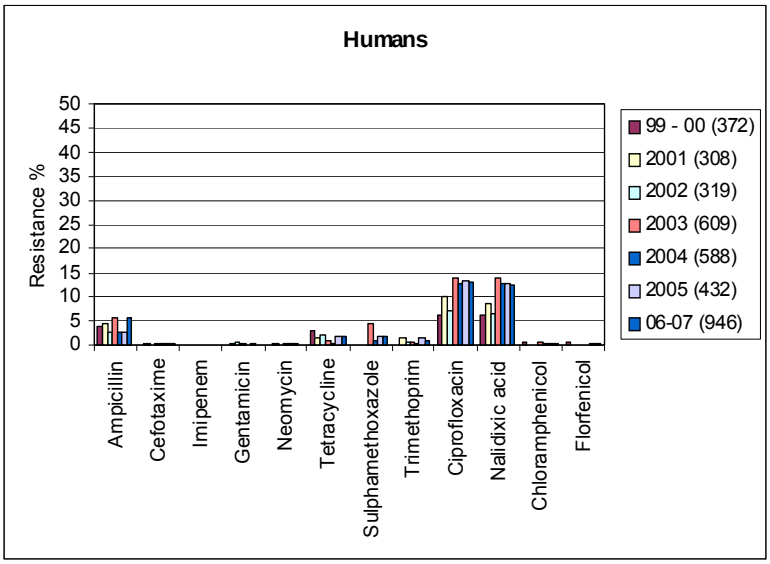
Table 12. Resistance (%) of *S.* Enteritidis and phagetypes 4, 21, 6, 1 and 8 isolated from different sources in 2006/2007.

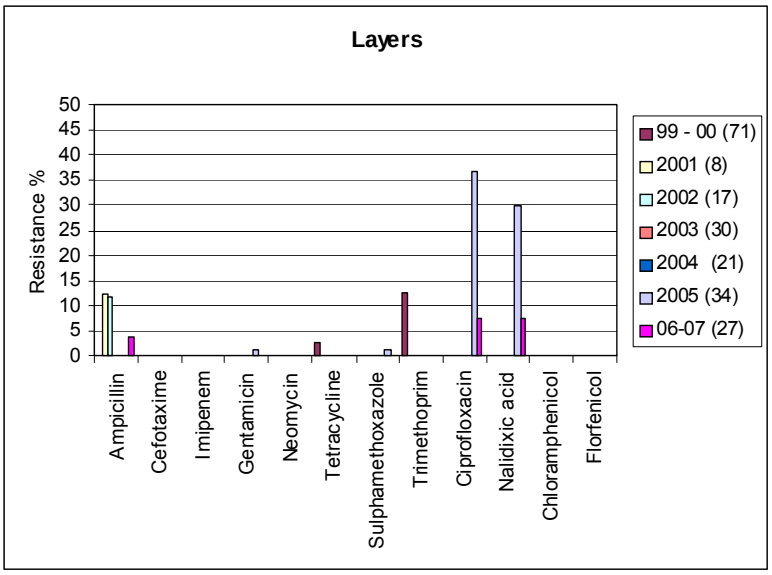
	S. Enteritidis			Most prevalent phage types				
	Human (946)	Layers (27)	Other poultry (26)	Pt4 (204)	Pt21 (148)	Pt6 (122)	Pt1 (97)	Pt8 (89)
Ampicillin	5.4	4	4	2	2	5.7	5.2	1.1
Cefotaxime	0.4	0	4	0	0	0	2	0
Ceftazidime	0.3	0	4	0	0	0	2	0
Gentamicin	0	0	0	0	0	0	0	0
Neomycin	0.1	0	0	0	0	0	0	0.0
Tetracycline	1.7	0	0	0	1	1	3	0
Sulphamethoxazole	1.5	0	0	1	1	0.8	5.2	0
Trimethoprim	1	0	4	0.7	1	0.8	2.1	0.0
Ciprofloxacin	12.9	7.4	42	6	10	3.3	64.9	3.4
Nalidixic acid	12.3	7.4	42.3	5.3	10.1	2	62.9	3
Chloramphenicol	0.4	0	0	0.2	0.0	0	3.1	0
Florfenicol	0.2	0	0	0	0	0	1	0
Streptomycin	0	-	-	0	0	0	0	0
Kanamycin	0.8	-	-	0	0	0	7.1	0
Colistin	0	-	-	0	0	0	0	0

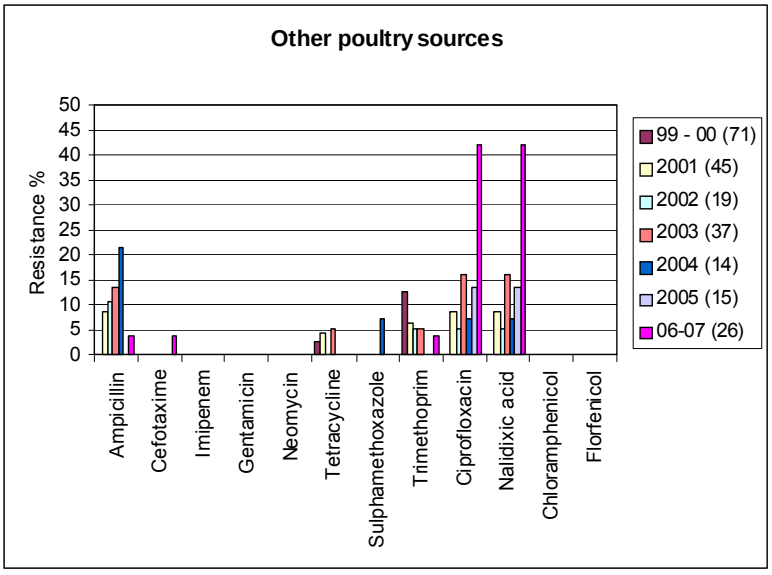
Resistance to the quinolones (ciprofloxacin and nalidixic acid) is stable in the last three years in isolates from humans (Fig. 10). The increase in cipro/Nal resistance % in other poultry sources in 2006/2007 is affected by the small numbers tested. These strains were mainly isolated from poultry meat.

It can be concluded that quinolone resistant strains of *S.* Enteritidis isolated from humans primarily originate from other sources than Dutch layers, like imported eggs, travel related infections and only to a lesser extend consumption of poultry meat products**.**

Figure 8. Trends in resistance (%) of *S.* Enteritidis isolated from humans, layers and other poultry sources from 1999 - 2007







S. Typhimurium

In 2006/2007 the most predominant phage types of *S.* Typhimurium in the collection of strains received from RIVM Bilthoven were: FT 506 (≈ DT104), FT 507 and FT 560 (table 13).

Although in *S.* Typhimurium resistance commonly present, resistance to quinolones occurs less frequently and ESBL suspected strains are rare, in comparison to other serovars**.** The most likely reason is that *S.* Typhimurium is more prevalent in pigs and cattle, where ESBLs are less common in the commensal gut flora and quinolones are used less frequently.

In 2006/2007 39 non ciprofloxacin wild type susceptible to ciprofloxacin *S.* Typhimurium isolates were found (MIC above the epidemiologic cut-off value), predominantly as human clinical isolates (69 %). Nineteen of these isolates were DT104 (38%). Although none of these isolates was high-level ciprofloxacin resistant, the reduced susceptibility to ciprofloxacin indicates a poor response to therapy with fluoroquinolones in case of invasive infections. This is an expert rule for the interpretation of MIC data on ciprofloxacin for Salmonella (www.eucast.org).

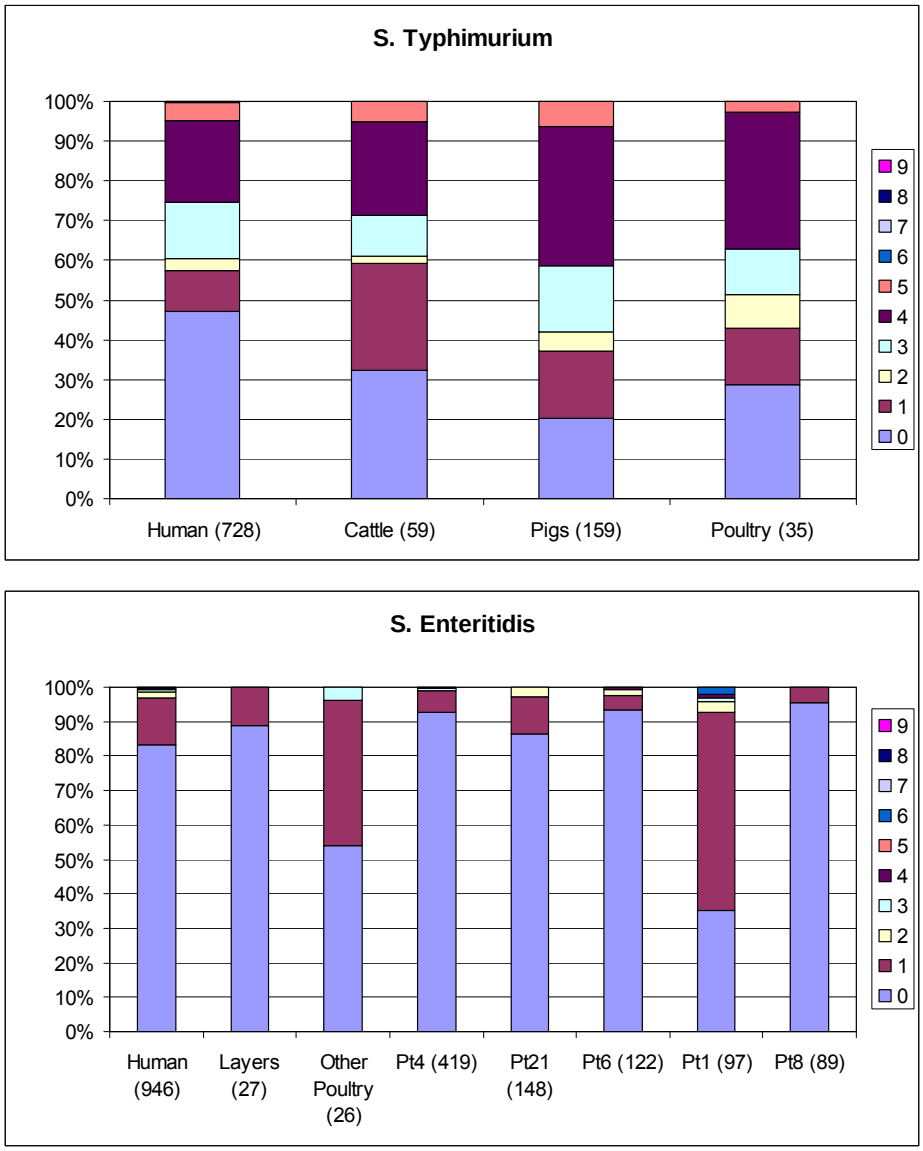
Resistance levels and multiple resistances are substantially higher in *S.* Typhimurium than in *S.* Enteritidis (Table 11, figures 8 and 10). Of the strains, 40% (humans),57%(pigs), 48% (poultry) and 39% (cattle) were resistant to three or more antibiotic classes (Fig. 9).

Resistance in *S.* Typhimurium shows a tendency to increase in strains from pigs (Fig. 12).
The relatively small number of the isolates per year and the differences in proportion of multi drug resistant phage types per category and per year affect the trend analysis**.**

Table 13. Resistance percentages of *S.* Typhimurium and phage types DT104, Ft 507, and FT560 isolated from different sources in 2006/2007.

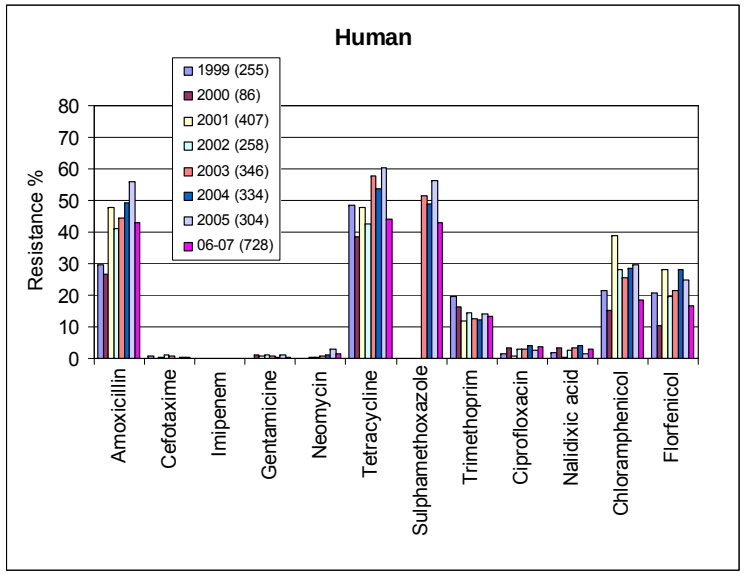
	S. Typhimurium				Phage types		
	Human (728)	Cattle (59)	Pigs (159)	Poultry (35)	DT104 (226)	Ft 507 (287)	FT 560 (187)
Ampicillin	42.9	55.9	57.2	57.1	83.6	60.6	3.7
Cefotaxime	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gentamicin	0.5	0.0	0.0	0.0	0.4	0.0	0.0
Neomycin	1.6	0.0	0.7	11.4	0.0	4.3	0.0
Tetracycline	44.1	45.8	69.2	60.0	81.9	63.1	3.7
Sulphamethoxazole	43.1	42.4	64.2	57.1	91.6	55.4	5.3
Trimethoprim	13.2	11.9	28.9	14.3	8.8	24.7	4.8
Ciprofloxacin	3.7	1.7	2.5	2.9	8.8	3.1	2.1
Nalidixic acid	3.0	1.7	1.9	2.9	8.8	2.4	0.0
Chloramphenicol	18.5	23.7	30.8	25.7	77.4	4.5	0.0
Florfenicol	16.5	22.0	28.3	25.7	77.0	1.0	0.0
Streptomycin	43.5	-	37.5	-	90.0	44.8	-
Kanamycin	4.3	-	0	-	0	10.3	-
Colistin	0	-	0	-	0	0	-

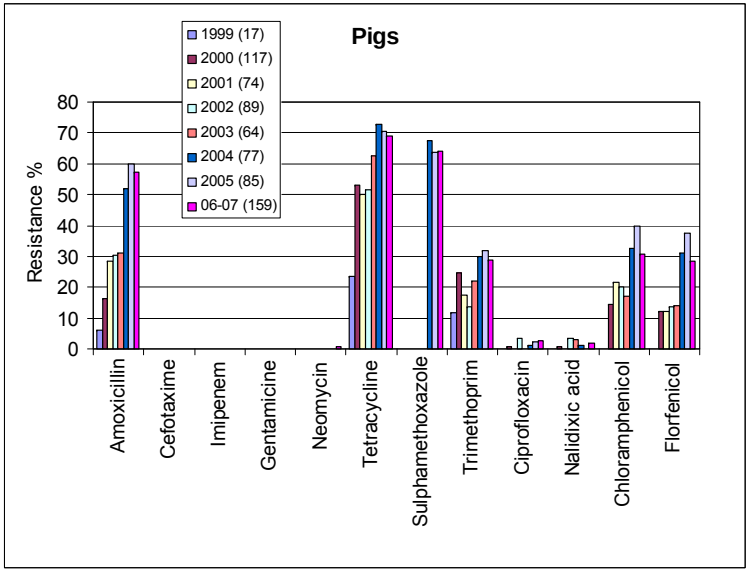
Figure 9. Percentages of *S.* Typhimurium and *S.* Enteritidis strains fully susceptible, resistant to one to nine different antibiotic classes in human and animal sources in The Netherlands in 2006/2007.

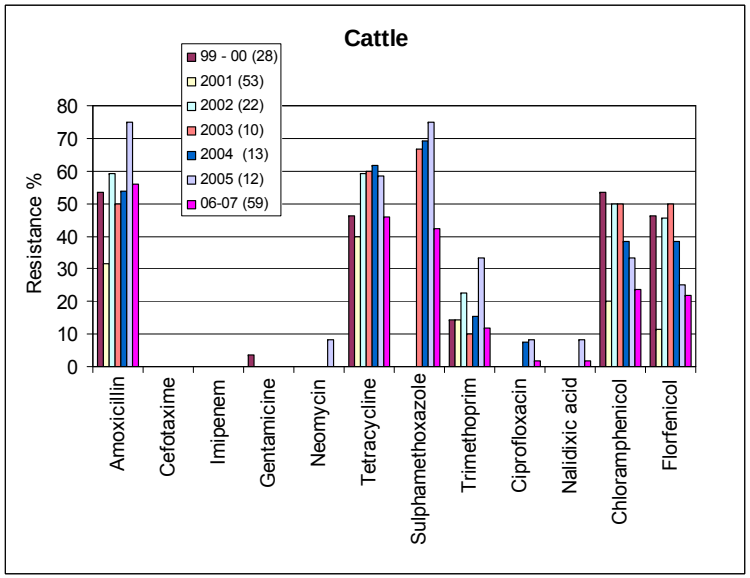


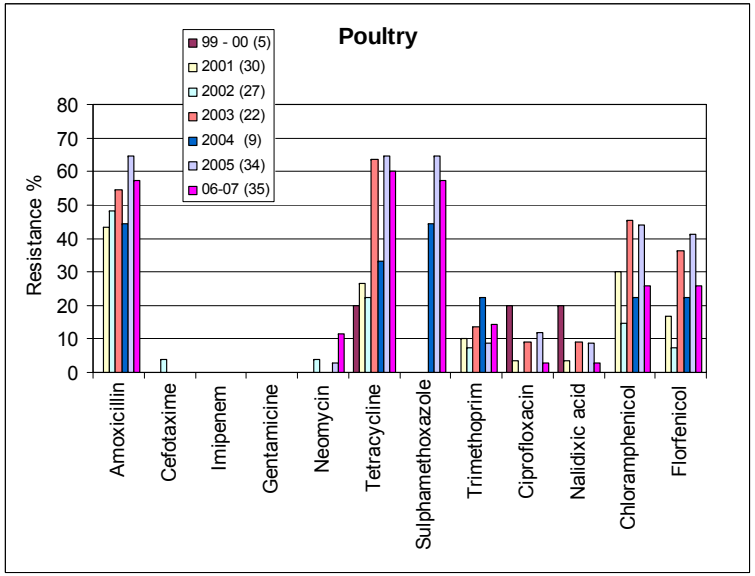
Multi drug resistance is more common in Typhimurium compared to Enteritidis, which is predominantly caused by the frequent occurrence of multiresistant phage types like DT104 (fig. 11). In Enteritidis resistance is mostly limited ton one or two antibiotic classes**.** In Pt1 the highest incidence of multiresistance is observed. This phage type is related to the Iberian peninsula, which may explain the higher resistance percentages**.**

Figure 10. Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals from 1999 - 2007









S. Paratyphi B var. Java (*S.* Java)

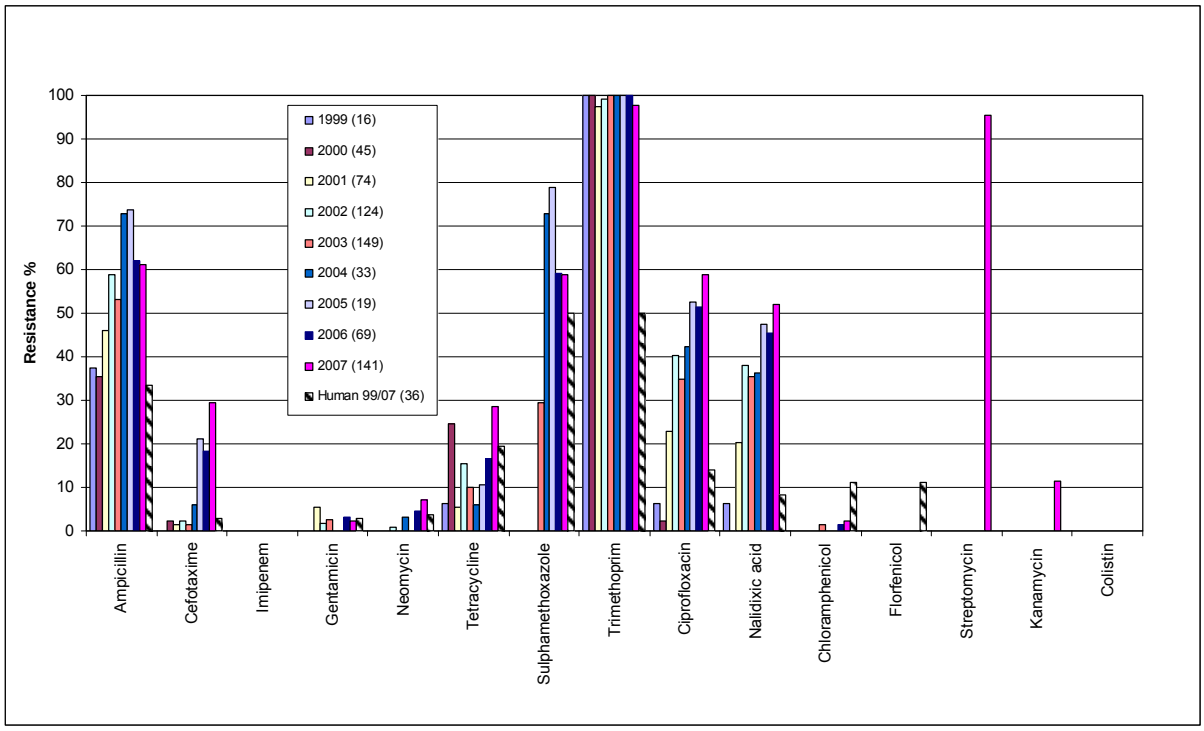
The prevalence data from the Dutch salmonella reference laboratory (RIVM) in the 2006/2007 show that *S.* Java in 2006/2007 was still the most predominant serovar in broiler production. This is confirmed by the isolation rate of 63% of this serovar from poultry products in 2007.
In 2006/2007 12 *S.* Java were isolated from a human infections**.** The majority were fully susceptible to all antibiotics in the panel and therefore not related to the clone spreading in Dutch poultry. However, one isolate was ESBL positive and also high level resistant to fluoroquinolones (Cipro MIC 2 mg/L). From poultry 129 strains were isolated of which 98% harboured the phenotype typical for the clone.

Non wild type susceptibility to ciprofloxacin in *S.* Java isolated from poultry increased to 58% (N = 76). In 2006/2007 for the first time 6 isolates were found with high level resistance to ciprofloxacin.

Resistance to cefotaxime (ESBL-producers) shows a clear increase in 2006/2007. This is related to the increase in ESBLs in commensal *E. coli* from broilers, by horizontal transfer of plasmid mediated beta-lactamases. Third-generation cephalosporins are not used in broiler production, but the use of ceftiofur in combination with Marek vaccine or with in ovo vaccination is a common procedure in the poultry reproduction and breeding sectors**.** It is likely that this has contributed to selection and vertical transmission of ESBLs in the poultry production pyramid.

Resistance to tetracycline shows a similar tendency to increase as observed for cefotaxime. This indicates acquisition of tet-genes in the Java clone in The Netherlands.
In 2006/2007 we included streptomycin in the panel of antibiotics. Virtually all poultry isolates were resistant, which is related to the aadA1-gene in the class-2 integron typical for the clone.

Figure 11. Trends in resistance (%) of *S.* Paratyphi B var. Java isolated from poultry from 1999 – 2007 and humans (Grey-white dashed bars indicate all humans S. Java isolates from 1999 – 2007 (N = 36))



Salmonella in raw meat products of food-animals at retail

Table 14. Resistance (%) of *Salmonella enterica* isolated from raw meat from poultry, and other raw meat sources in 2006/2007.

	Poultry S. Java N = 46	Poultry other serovars N = 96	Other Raw meat sources N = 35
Ampicillin	61	42	34
Cefotaxime	9	3	0
Ceftazidime	10	4	0
Gentamicin	0	4	3
Tetracycline	22	28	43
Sulfamethoxazole	61	47	60
Trimethoprim	98	41	20
Ciprofloxacin	63	44	9
Nalidixic acid	61	43	11
Chloramphenicol	2	6	29
Florfenicol	0	3	11
Streptomycin	26	29	30
Kanamycin	10	14	7
Colistin	0	0	0

In 20056/2007, as in previous years, in raw meat products originating from poultry *S.* Java was still by far the most prevalent serovar isolated. Almost all *S.* Java's were resistant to trimethoprim, typical for the clone. Resistance levels for the quinolones were similar as observed in isolates from broilers**.** Resistance to cefotaxime was less predominant than in isolates from live animals**.**
Resistance trends are presented for poultry products only, because in beef and pork the numbers of isolates examined are too small to provide an accurate estimate (Fig. 12). The variable contribution of *S.* Java to the annual resistance percentages over all serotypes hampers the interpretation of the observed trend in the resistance.

Figure 12. Trends in resistance (%) of *Salmonella enterica* isolated from poultry products in the Netherlands from 2001 – 2007.

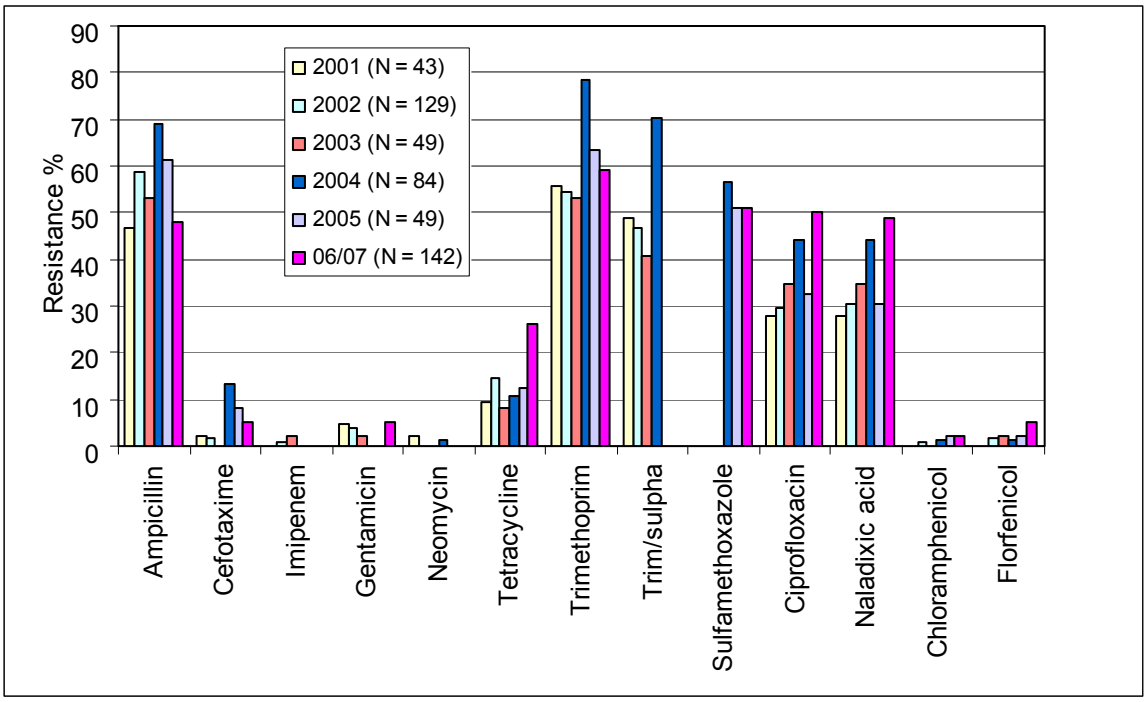


Table 15. Distribution of *Salmonella* serovars, in poultry meat at retail (Surveillance data of Food and Consumer Product Safety Authority (VWA-KvW)) from 1997 tot 2007

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
N	1314	1077	859	1454	1578	1600	1510	1482	1474	1410	1395
Salmonella spp. positive (%)	29.1	20.2	17.6	21	16.3	13.4	11.3	7.4	9.4	8.4	8.2
Main serovars as a fraction of all isolates (%)											
Paratyphi B var. Java	15.0	11.4	13.9	33.1	43.2	53.5	45.6	58.2	46.8	38.5	63.2
Enteritidis	20.2	12.8	26.4	6.6	8.2	2.3	8.8	5.5	7.2	6.6	1.8
Hadar	10.1	6.1	4.5	3.3	4.2	0.9	1.8	-	1.4	5.7	-
Indiana	6.1	8.3	9.3	10.2	11.6	6.5	6.4	1.8	2.2	4.1	5.3
Infantis	9.2	5.0	3.6	6.6	7.0	7.9	11.7	-	11.5	13.9	10.5
Virchow	4.6	2.8	2.6	10.2	3.5	5.6	5.8	4.5	8.6	11.5	3.5
Typhimurium (DT104)	7.8	3.6 (1.8	1.3 (0.7)	0.1 (0.1)	7.4 (7)	7.4 (2.8)	5.8 (5.3)	3.6	5 (2.2)	1.6	0.9
Corvallis									4.3	1.6	-
Other types	27.0	53.6	39.7	30.0	22.3	23.3	19.9	26.4	18.0	16.5	14.8

Salmonella in animal feeds, turkeys, horses, ducks, pigeon and reptiles

Table 16 presents the most prevalent serovars found in animal feeds from 2001 – 2007 per single and or compound feed type. Moreover R% of Salmonella strains isolated from incidental animal sources are presented. The serotypes Senftenberg, Agona, Lexington, Mbandaka and Rissen are most frequently isolated from animal feeds**.** Resistance in these serovars is very uncommon, except tetracycline resistance.

In salmonella's isolated from turkeys, horses and ducks, more resistance was observed than in strains from pigeons or reptiles**.** Nalidixic acid and ciprofloxacin resistance was highest in turkeys and ducks, animals with a substantial consumption of quinolones (only fluoroquinolones are licensed for use in Turkeys).

Table 16. The most prevalent serovars isolated from animal feed and resistance (R%) of isolates of *Salmonella enterica* per single and or compound feed type, in 2001 – 2007. Moreover R% of *Salmonella* strains isolated from incidental animal sources over 2001 – 2007 are presented.

			Fish meal (56)	Animal meal (65)	Soy (feed, N=952)	Rapeseed (feed, N=343)	Single feed, other (339)	Composite feed (128)	Feed 2006-2007, N=470	Feed 2001-2005, N=1414	Turkey (94)	Horse (45)	Duck (11)	Pigeon (45)	Reptilian/Amfibian (69)
Serovar	N														
Senftenberg	255	Antibiotics	%-resistant isolates 2001-2007						%R		%-resistant 2001-2007				
Agona	211	ampicillin	0	1.5	0.5	0.9	1.8	1.6	0.6	1.0	44.7	20.0	9.1	11.1	1.4
Mbandaka	183	cefotaxime	0	0	0.2	0	0	0.8	0.4	0.1	2.1	0	0	0	0
Lexington	169	ceftazidime	0	0	0.3	0	0	0	0.2	0	2.9	0	0	0	--
Rissen	132	imipenem	0	0	0	0	0	0		0	0	0	0	0	0
Cubana	125	gentamicin	0	0	0	0	0.3	0	0.0	0.1	13.8	0	0	0	0
Livingstone	93	neomycin	0	0	0.2	0	0.6	0	0.9	0	14.9	0	0	0	0
Tennessee	85	tetracycline	0	1.5	0.8	0.3	1.8	5.5	0.6	1.4	42.6	17.8	9.1	6.7	2.9
Anatum	77	trim/sulpha	0	0	0.5	0	0.8	0		0.4	0.0	33.3	--	0.0	0.0
Havana	64	sulphamethoxazole	0	0	0.5	2.1	2.4	4.8	0.9	1.7	38.0	11.1	0	9.1	--
Kentucky	56	trimethoprim	0	1.5	0.4	0.9	0.6	2.3	0.6	0.7	12.8	17.8	0	0	0
Oranienburg	41	ciprofloxacin	1.8	1.5	0.2	0	0.9	3.1	1.1	0.4	46.8	2.2	18.2	0	0
Montevideo	37	nalidixic acid	0	1.5	0.1	0	0.9	2.3	0.9	0.3	40.4	2.2	18.2	0	0
Infantis	36	chloramphenicol	0	1.5	0.6	0.9	2.7	3.1	1.5	1.1	6.4	15.6	0	11.1	1.4
Minnesota	34	florfenicol	0	0	0.5	0.9	0.9	0	0.6	0.6	4.3	6.7	0	11.1	1.4
Cerro	32	streptomycin	--	--	4.2	--	10.0	--	5.6	--	66.7	--	--	--	--
Yoruba	29	kanamycin	--	--	8.3	--	20.0	--	11.1	--	83.3	--	--	--	--
17 main serotypes	1659 (83%)														
All serotypes	1988														

Campylobacter spp.

Highlights
In *C. jejuni* resistance levels for ciprofloxacin and nalidixic acid were substantially higher than in previous years, in particular in broilers**.** At the same time in human isolates a substantial increase in resistance to fluoroquinolones was observed. In 2007 for the first time an erythromycin resistant *C. jejuni* strain was isolated from a cow. Erythromycin resistance is however a regular finding in humans to some extent travel related or related to consumption of contaminated imported products, or due to human therapeutic use of macrolides
In *C. coli* resistance to macrolides occurred frequently. In 2006/2007 for the first time next to erythromycin, the related antibiotics clarithromycin and tulathromycin were included in the tests**.** The resistance levels were slightly different from erythromycin, which is probably due to the cut-off values used.

Table 17. MIC distribution (in %) for all C. jejuni (N = 182, of which 60 from broilers, 53 from dairy cows, 1 from pigs and 68 from veal calves) and C. coli (N = 320, of which 143 from pigs, 32 from broilers, 10 from cattle and 135 from veal calves) isolated from poultry, pig and cattle faeces in The Netherlands in 2006/2007.

C. jejuni	MIC (%) distribution															
(N = 182)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin			0.5	3.3	4.9	50.5	18.1	2.2	3.3	17.0						20.3
Gentamicin		91.2	8.2	0.5												0.0
Neomycin			69.2	22.0				2.2	4.4	1.6	0.5					8.8
Strepomycin				95.1	1.6		0.5	0.5	1.6			0.5				3.3
Tetracycline			47.3	1.1	2.7	1.1		0.5	1.6	12.6	33.0					48.9
Trim/sulphamethoxazole				6.2	46.2	38.5	4.6		3.1	1.5						4.6
Sulphamethoxazole							10.4	8.2	18.1	41.8	13.7	3.8	2.2	1.6		3.8
Ciprofloxacin	46.2	12.1	4.4			1.1	7.7	17.6	11.0							37.4
Nalidixic acid				0.5	14.3	38.5	7.7	1.6		0.5	8.8	28.0				37.4
Erythomycin			11.5	33.0	38.5	14.8	1.6				0.5					2.2
Clarithromycin			13.7	26.5	44.4	14.5	0.9									0.0
Tulathromycin			65.8	32.5	1.7											0.0
Chloramphenicol					30.8	54.9	8.8	5.5								0.0
C. coli	MIC (%) distribution															
(N = 320)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin			1.6	5.3	8.1	26.6	28.8	19.4	2.2	8.1						29.7
Gentamicin		59.7	30.9	6.9		0.3				2.2						2.5
Neomycin			48.1	34.1	0.9	0.6	1.6	0.3	2.2	3.8	8.4					16.9
Strepomycin				29.4	5.9	0.9	3.4	15.3	21.6	12.2	2.8	8.4				63.8
Tetracycline			10.0	2.8	0.6		0.3		0.6	9.1	76.6					86.6
Trim/sulphamethoxazole		0.8	3.4	21.0	29.4	6.7		5.9	22.7	10.1						38.7
Sulphamethoxazole							12.5	12.5	18.4	7.8	0.9	2.8	23.8	15.3	5.9	45.0
Ciprofloxacin	40.0	14.1	2.5	0.3	0.3	1.6	9.1	17.8	14.4							43.1
Nalidixic acid					2.2	28.1	23.1	3.4	0.6	5.0	19.1	18.4				43.1
Erythomycin			4.7	10.9	17.8	34.7	11.9	1.3	1.6	0.3	16.9					18.8
Clarithromycin			8.0	13.9	21.4	30.3	8.5	1.5	0.5		15.9					17.9
Tulathromycin			35.8	35.4	7.5	3.3	0.5	0.5	0.9	4.2	11.8					17.0
Chloramphenicol					5.5	34.0	51.8	7.8	0.6	0.3						1.0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values, used as breakpoints**.**

Table 17 presents the MIC-distributions and resistance percentages for all Campylobacter jejuni and *C. coli* strains isolated in 2006/2007. In *C. jejuni* highest resistance levels are observed for tetracycline and the quinolones**.** In 2007 for the first time an erythromycin resistant *C. jejuni* strain was isolated from a cow. *C. coli* showed much more resistance and at higher levels than *C. jejuni*.

In *C. jejuni* from poultry the resistance levels in isolates from broiler faeces and from poultry raw meat products sampled by the Dutch Food Safety Authority at retail are very similar except for sulphamethoxazole, for which the levels were much higher in isolates from products (table 18). This was expected for isolates from broiler faeces and poultry raw meat products because they should represent the same bacterial populations**.** In *C. jejuni* resistance levels for ciprofloxacin and nalidixic acid were substantially higher that in previous years**.** This may be caused by a true increase of resistance or by selection bias. In 2006/2007 the samples from which campylobacter was isolated were partly taken as part of the national control program and partly as part of the EU-baseline study in poultry. In isolates from cattle the resistance levels reflect the use patterns of antibiotics**.** Tetracycline and to a lesser extend quinolone resistance is highest in veal calves**.**

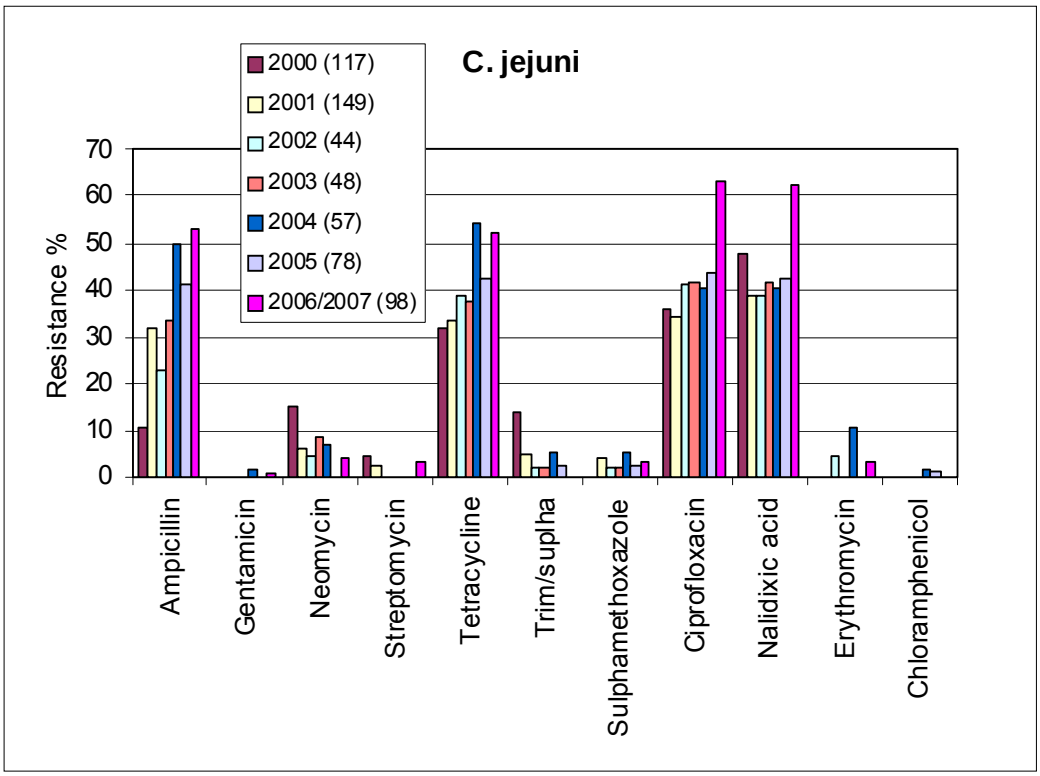
In *C. coli* resistance to macrolides occurred frequently. In 2006/2007 for the first time next to erythromycin, the related products clarithromycin and tulathromycin were included in the tests**.** The resistance levels were slightly different from erythromycin, which is probably due to the cut-off values used.

Table 18. Resistance percentages of *C. jejuni* and *C. coli* isolated from broilers, raw meat products from poultry in 2006/2007

	Broilers		Poultry products		Pigs	Veal calves		Dairy cows	
	C. jejuni	*C. coli*	*C. jejuni*	*C. coli*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*
N	98	32	156	106	143	68	135	53	10
Ampicillin	53.1	40.6	43.0	26.2	16.8	13.2	38.5	0	60
Gentamicin	1.0	0	0.6	0	0	0	5.9	0	0
Neomycin	4.1	3.1	7.4	2.9	4.9	14.7	34.1	3.8	0
Streptomycin	3.1	0	1.3	21.4	75.5	5.9	68.9	1.9	30
Tetracycline	52.0	53.1	42.3	59.2	86.7	80.9	98.5	7.5	30
Trim/sulphamethoxazole	13.3	6.3	-	-	55.0	3.4	34.4	0	25
Sulphamethoxazole	3.1	6.3	31.5	12.6	55.9	1.5	43.7	5.7	30
Ciprofloxacin	63.3	75.0	43.6	67.0	9.8	39.7	72.6	13.2	20
Nalidixic acid	62.2	78.1	42.3	66.0	9.8	39.7	71.9	13.2	20
Erythromycin	3.1	9.4	0.7	7.8	21	2.9	19.3	1.9	10
Clarithromycin	0	14.3	0.7	7.8	15.5	0	18.3	0	0
Tulathromycin	0	14.3	0.7	7.8	18.4	0	19.7	0	0
Chloramphenicol	0	0	0	0	0	0	2.2	0	0

In *C. jejuni* from broilers resistance levels of ampicillin, tetracycline and the quinolones have increased since 2000. In *C. coli* from pigs the resistance levels are stable except resistance to erythromycin, which increased again after a decrease in the past years**.** This may be related to increased therapeutic usage of macrolides (tylosin, mycotil) (fig 13). The resistance data in table 18 reflect the use patterns of the antibiotics in different animal species**.** The quinolone resistance levels are highest in veal calves and broilers, the animals in which this drug class is predominantly used.

Figure 13. Trends in resistance (%) of *C. jejuni* isolated from broilers and *C. coli* isolated from slaughter pigs and broilers (grey striped bars), from 2000 - 2007 in The Netherlands



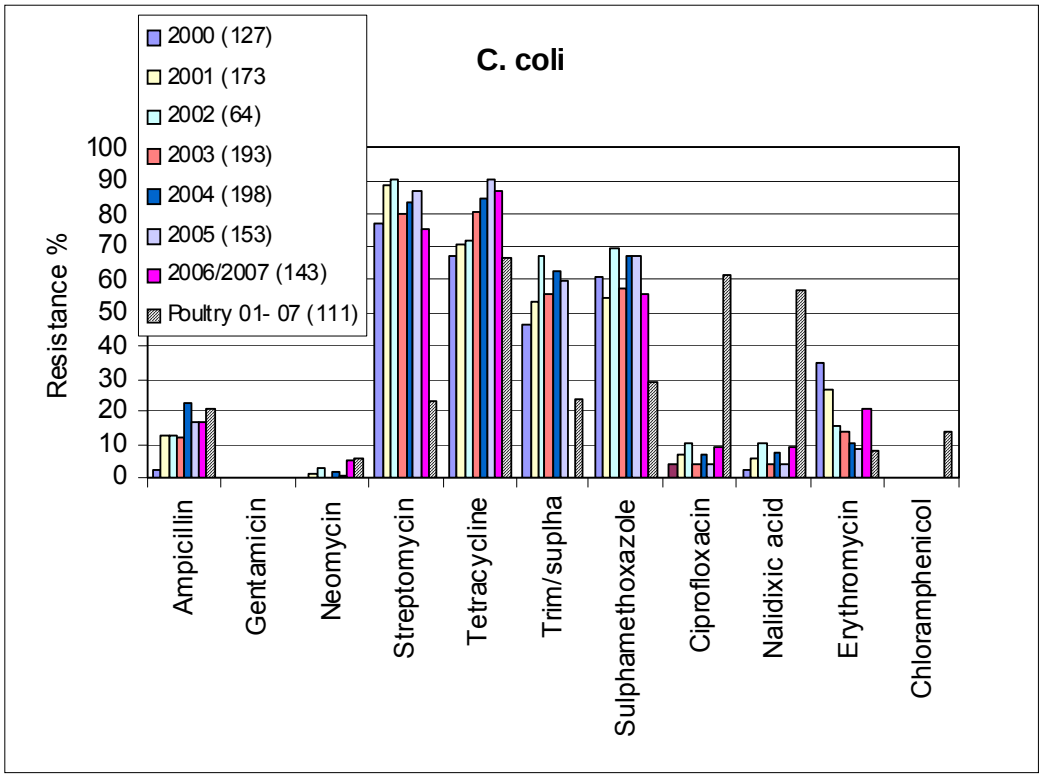
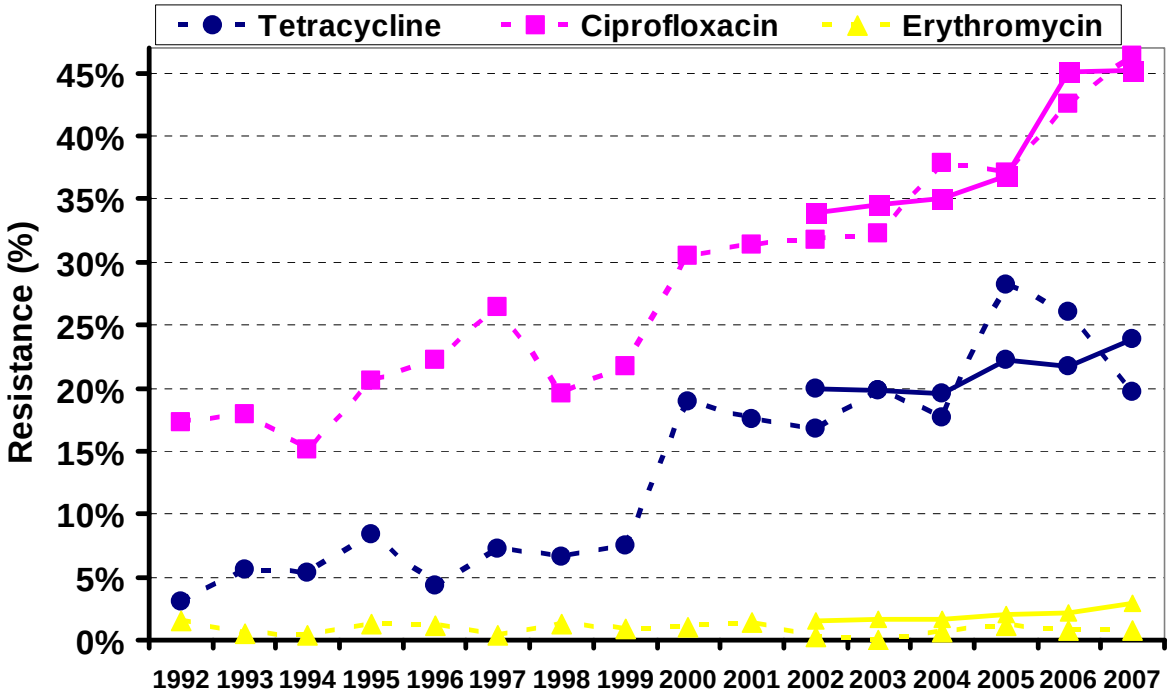


Figure 14. Trends in resistance (%) of *Campylobacter* spp. isolated from humans isolated between 1992 and 2007 at the regional Public Health Laboratories (PHLS) of Arnhem and Heerlen covering 990.000 inhabitants. The dotted line represents data from the national surveillance in 1992 – 2007, the continuous line represents national surveillance data; annually the average number of strains tested was approximately 2400, ranging from 1900 – 2900.



In *Campylobacter* spp. isolated from humans, resistance to fluoroquinolones has again increased., like in broilers, in particular between 2005 and 2006. Resistance to erythromycin remains rare (fig. 14). Table 19 shows that as was found in previous years, in travel-related infections fluoroquinolone resistance occurred more frequently than in isolates from domestically acquired infections, for tetracycline this difference was observed for *C. jejuni* only.

Table 19. Domestically acquired and travel related resistance in *C. jejuni* and *C. coli* isolated from humans from 2002 - 2007 from all 16 PHLS covering > 50% of the Dutch population.

	2002-2005								2006-2007							
	Domestically acquired				Travel related				Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*		*C. jejuni*		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	6831	32,7	389	36	601	53,4	57	49,1	4763	43,7	327	42,5	269	63,6	32	68,8
Tetracycline	5075	18,5	355	22,5	430	26,7	49	20,4	3262	21,5	257	26,5	154	29,2	31	19,4
Erythromycin	5765	1,2	374	2,9	511	1,6	53	0	3784	1,8	282	5	212	3,8	29	10,3

In *C. jejuni* strains isolated from Dutch poultry until 2005 not one high level erythromycin resistant strain has been detected. Therefore human infections with *C. jejuni* strains resistant to erythromycin may be travel related or related to consumption of contaminated imported products, or due to human therapeutic use of macrolides.

Shigella toxin producing *E. coli* O157

Highlights
Resistance in *E. coli* O157 was traditionally rarely present. In human isolates resistance levels were low. In isolates from calves however, resistance was quite commonly present, with levels varying from 0.7 – 20.7 %. In calves one ESBL suspected isolate was detected.
No clear trends in resistance can be observed. The levels vary from year to year.

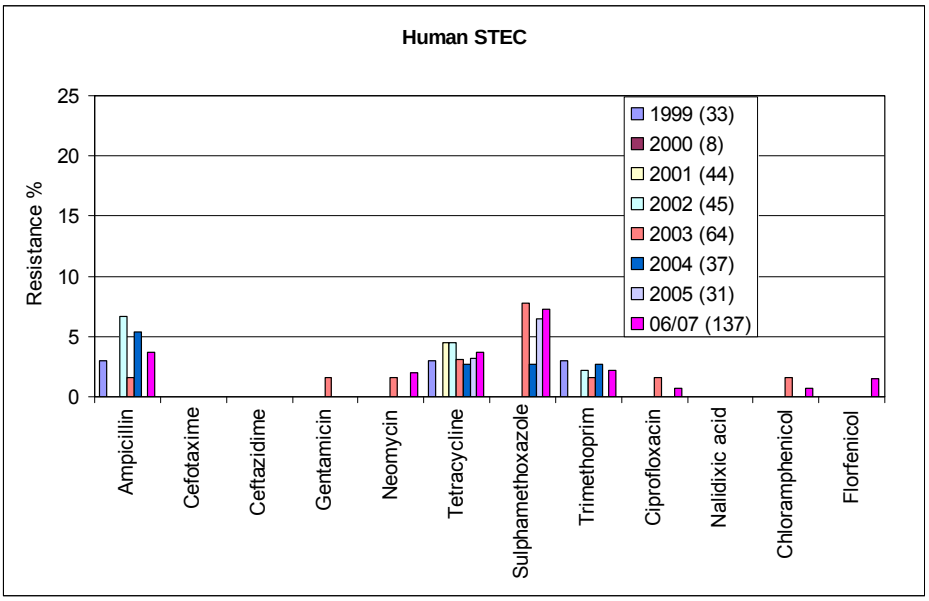
Table 20. MIC distribution (in %) for *E. coli* O157 isolated in The Netherlands in 2006/2007 from human (N = 137) and cattle faeces (N = 142)

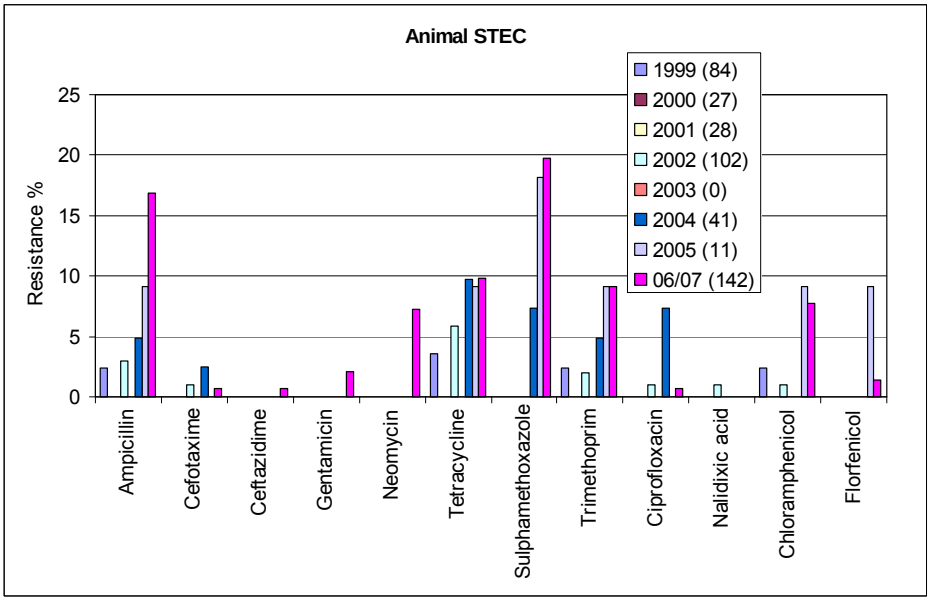
N = 137	MIC (%) distribution mg/L																		
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin								34.3	62.0				2.2	1.5					3.6
Cefotaxime			56.9	43.1															0
Ceftazidime				22.6	73.0	4.4													0
Gentamicin					20.4	64.2	13.1	2.2											0
Neomycin							91.8	4.1	2.0				2.0						2.0
Tetracycline							0.7	92.7	2.9				0.7	2.9					3.6
Sulphamethoxazole										92.7								7.3	7.3
Trimethoprim						97.8							1.5	0.7					2.2
Ciprofloxacin	6.6	57.7	35.0	0.7															0.7
Nalidixic acid								21.2	76.6	2.2									0
Chloramphenicol									16.1	80.3	2.9			0.7					0.7
Florfenicol									4.4	83.2	10.9			1.5					1.5
Streptomycin								2.3	80.7	10.2			1.1	2.3	3.4				6.8
Kanamycin									93.2	4.5	1.1				1.1				2.2
Colistin										100									0

N = 142	MIC (%) distribution mg/L																		
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin								23.2	59.2	0.7			12.7	4.2					16.9
Cefotaxime			50.7	48.6			0.7												0.7
Ceftazidime				23.9	73.9	1.4			0.7										0.7
Gentamicin					6.3	71.8	18.3	1.4			0.7	0.7	0.7						2.1
Neomycin							81.8	9.1	1.8			3.6			3.6				7.3
Tetracycline							0.0	76.1	14.1				2.1	7.7					9.9
Sulphamethoxazole										80.3								19.7	19.7
Trimethoprim						88.7	2.1						5.6	3.5					9.2
Ciprofloxacin	7.0	53.5	38.7	0.7															0.7
Nalidixic acid								28.2	70.4	1.4									0
Chloramphenicol									4.2	75.4	12.7		0.7	4.2	2.8				7.7
Florfenicol									8.5	81.7	8.5			0.7	0.7				1.4
Streptomycin									49.4	29.9			5.7	4.6	10.3				20.7
Kanamycin									85.1	6.9				1.1	6.9				8.0
Colistin										99		1.1							1.1

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the cut-off values used as breakpoints**.** Dashed bars indicate the clinical breakpoints**.**

Figure 15. Trends in resistance percentages of *E. coli* O157 (STEC) isolated in The Netherlands from 1998 - 2007





In 2006/2007, 279 isolates of notifiable Shi-toxin producing *E. coli* O157 (STEC) were sent to RIVM for routine typing. 137 specimens were taken from human and 142 from cattle faeces in an attempt to trace a human clinical infection (table 20).
Resistance in *E. coli* O157 was traditionally rarely present and limited to isolates from sporadic cases**.** In cattle isolates however, resistance was quite commonly present, with levels varying from 0.7 – 20.7 %. This includes one isolate demonstrating an ESBL-phenotype based on MICs for cefotaxime of 1 mg/L and ceftazidime 4 mg/L (table 20). The cattle isolates originated predominantly from calves (N = 117), which may explain the relatively high resistance levels**.**
In 2006/2007 resistance levels were higher than in previous years for most antibiotics tested. This could indicate a trend, but could also be due to annual variations**.** The most striking differences were observed for ampicillin and sulphamethoxazole. Streptomycin was tested for the first year (fig. 15). In general the resistance levels were higher in isolates from animals that from humans**.**

Commensal indicator organisms

The level of antimicrobial resistance in randomly sampled commensal organisms of the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics as therapeutics in animals, especially over time. For this purpose, *E. coli* and *Enterococcus faecium* and *E. faecalis*, as indicator organisms for the Gram-negative and Gram-positive flora, are monitored. Isolation of bacteria from the intestine of randomly picked animals at slaughter aims to detect the development of resistance at the bacterial population level in food animals**.**
In 2005 we started to monitor resistance in isolates from both dairy cattle and veal calves. For this purpose we used the samples that were taken at farms to determine the prevalence of *Salmonella, E. coli* O157 and *Campylobacter*.
Resistance percentages in tables 21, 23 and 24 indicate the level of resistance in all *E. coli*, *E. faecium* and *E. faecalis* strains of slaughter pigs, broilers, dairy cows and veal calves, respectively. Because of the sampling strategy, this method is inherently insensitive for detecting resistance. The method is insensitive because only one randomly selected isolate per epidemiological unit (herd or flock) is selected. The total sample of selected isolates is intended to represent the *E. coli*, or *Enterococcus* species population of each animal species of the entire Netherlands**.** One percent resistance in eg. *E. coli* indicates that in all animals 1% of the *E. coli* bacteria are resistant. Because each animal harbours app. 106 cfu/g faeces *E. coli* in its gut, 1% would be app. 104cfu/g faeces**.** This means that when no resistance is detected, this does not exclude the possibility that with selective enrichment resistance could be detected.

Escherichia coli

Highlights
Overall, the resistance levels of food-borne commensal *E. coli* were highest in broilers and poultry raw meat products, followed by veal calves, slaughter pigs and dairy cattle, in which resistance is rare. In broilers and slaughter pigs, for most antibiotics the resistance levels show a tendency to increase. This increase is also obvious for multi-drug resistance.
In broilers and poultry raw meat products resistance to cefotaxime indicative of extended spectrum beta-lactamases (ESBLs), has increased from 9.7% in 2004 to almost 17% in 2006/2007.

Traditionally in *E. coli* isolated from food-producing animals resistance to ampicillin, tetracycline, sulphamethoxazole and trimethoprim is very commonly present. In 2006/2007 for the first year streptomycin was included, which resulted in high resistance levels in all animals studied. (table 21). Overall, the resistance levels were highest in broilers, followed by veal calves, slaughter pigs and dairy cattle, in which resistance is rare.
In broilers resistance to ciprofloxacin and nalidixic acid are stable at app. 50%. However, high level ciprofloxacin resistance occurred in 6.5% of the isolates, which is substantially more than in 2006 (2.6%)
In broilers and slaughter pigs in particular, for most antibiotics the resistance levels show a tendency to increase, while in cattle the levels seem more stable (fig 17). This increase is also obvious for multi-drug resistance, although the situation seems to stabilize somewhat in 2006/2007 at a high level except in dairy cattle where multi-drug resistance is rare.
In broilers resistance to cefotaxime (and ceftazidime that was only tested in 2007), indicative of the presence of extended spectrum beta-lactamases (ESBLs), has increased from 9.7% in 2004 to almost 17% in 2006/2007. The synchronous increase in cefotaxime resistance in Salmonella from poultry indicates transfer of plasmid mediated ESBLs between these species**.**
The observed increase in chloramphenicol resistance demonstrates the complexity of the current resistance situation in broiler (fig 17). Chloramphenicol has been banned and not been used in almost 20 years and still resistance increases**.** This is the result of integrons and plasmids on which chloramphenicol-resistance genes (catA) are linked to a.o. beta-lactamases, tetracycline- and sulphonamide resistance genes**.**

Table 21. MIC distributions (in %) for *E. coli* isolated as indicator organism from intestines of slaughter pigs (N = 248), broiler chickens (N = 197), veal calves (N = 327) and dairy cattle (N = 280) in The Netherlands in 2006/2007.

Slaughter pigs	MIC (%) distribution mg/L																	
N = 248	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin							3.6	30.6	29.4	0.4		0.4	11.3	24.2					35.9
Cefotaxime			26.6	72.2	0.4	0.4		0.4											0.8
Ceftazidime				49.6	48.0	1.6		0.8											0.8
Gentamicin					6.0	52.0	31.5	6.0	2.8		0.4	0.8	0.4						4.4
Neomycin							61.7	24.6	9.6	1		1.2	1.8	0.6					3.6
Tetracycline							6.5	13.7	7.3	0.8	2.0	3.6	25.4	40.7					71.8
Sulphamethoxazole										45.2	0.4						2.0	52.4	54.4
Trimethoprim						47.6	2.4	0.4					16.1	33.5					49.6
Ciprofloxacin	17.7	13.3	66.5	0.8	1.2	0.4													2.4
Nalidixic acid								54.8	41.9	1.2		0.4		1.6					2.0
Chloramphenicol									10.5	71.4	6.5	3.2	3.6	2.8	2.0				11.7
Florfenicol								0.4	22.6	68.5	8.1				0.4				0.4
Streptomycin									17.3	25.9	4.9	7.4	6.2	7.4	30.9				51.9
Kanamycin									60.5	25.9	8.6	1.2			3.7				13.6
Colistin										100									0.0

Broilers	MIC (%) distribution mg/L																	
N = 197	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin						0.5	3.6	17.3	13.2	1.0			13.7	50.8					64.5
Cefotaxime			15.2	68.0		0.5			0.5	7.1	2.5	6.1							16.8
Ceftazidime				42.1	40.1	1.5	4.1	3.6	0.5	2.5	5.1	0.5							16.2
Gentamicin					4.1	48.2	35.5	5.6		1.5	2.0	2.5	0.5						6.6
Neomycin							59.7	21.4	2.6			2.6	7.1	3.9	2.6				16.2
Tetracycline							12.7	21.3	12.7				19.8	33.5					53.3
Sulphamethoxazole										31.0							3.0	66.0	69.0
Trimethoprim						37.6	2.5	1.0					11.2	47.7					58.9
Ciprofloxacin	3.0	8.1	39.1	5.1	27.4	8.6	2.0		0.5	2.0	1.0	3.0							49.7
Nalidixic acid								29.9	18.8	1.0	0.5	2.5	8.1	20.8	18.3				49.7
Chloramphenicol									21.3	68.5	9.1	0.5			0.5				1.0
Florfenicol									6.1	66.0	9.6	3.0	2.5	2.0	10.7				18.3
Streptomycin									7.0	23.3	18.6	4.7	4.7	4.7	37.2				51.2
Kanamycin									62.8	25.6	4.7				7.0				11.6
Colistin										100									0.0

Veal calves	MIC (%) distribution mg/L																	
N = 327	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin							0.9	19.3	28.7	3.7	0.3	0.6	9.8	36.7					47.4
Cefotaxime			17.4	74.6	4.6	1.5	0.9	0.3		0.3		0.3							3.4
Ceftazidime				40.7	50.5	6.1	1.2		0.6	0.9									2.8
Gentamicin					4.9	46.2	34.3	5.8	0.9	0.6	2.8	1.8	2.8						8.9
Neomycin							56.0	15.9	3.2	1		2.4	6.0	8.3	7.1				23.8
Tetracycline							3.1	18.0	7.6	0.3		0.9	15.3	54.7					70.9
Sulphamethoxazole										47.2							1.5	51.2	52.8
Trimethoprim						56.3	0.9				0.3		10.4	32.1					42.8
Ciprofloxacin	9.2	9.8	63.6	0.9	3.7	3.7	1.2	0.9		0.6	3.7	2.8							17.4
Nalidixic acid								42.8	38.2	1.5	0.3		1.2	5.2	10.7				17.1
Chloramphenicol									5.8	55.4	11.0	2.1	3.1	7.3	15.3				27.8
Florfenicol								0.9	11.3	63.0	15.0	2.1	0.6	1.5	5.5				9.8
Streptomycin								1.3	18.7	22.7	5.3	5.3	6.7	8.0	32.0				52.0
Kanamycin									60.0	16.0	1.3				22.7				24.0
Colistin										100									0.0

Dairy cattle	MIC (%) distribution mg/L																	
N = 280	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin							3.2	28.9	58.6	5.4	0.4		2.1	1.4					3.9
Cefotaxime			21.1	77.1	1.4							0.4							0.4
Ceftazidime				51.8	45.4	2.5		0.4											0.4
Gentamicin					5.0	66.1	24.6	3.9	0.0	0.0	0.4								0.4
Neomycin							77.0	18.3	3.3	0			0.5	0.5					0.9
Tetracycline							10.7	59.3	24.6	0.4		0.4	1.4	3.2					5.0
Sulphamethoxazole										95.0							0.4	4.6	5.0
Trimethoprim						93.6	1.8	0.4		0.4			1.8	2.1					4.3
Ciprofloxacin	9.6	13.9	76.1									0.4							0.4
Nalidixic acid								62.9	35.4	1.1		0.4			0.4				0.7
Chloramphenicol									3.2	80.7	14.6		0.4	0.4	0.7				1.4
Florfenicol								0.4	12.1	81.4	5.4	0.4		0.4					0.7
Streptomycin									31.3	52.2	4.5	1.5	1.5	3.0	6.0				11.9
Kanamycin									80.6	14.9	4.5								4.5
Colistin										100									0.0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values used as breakpoints**.** The dashed bars indicate the clinical breakpoints**.**

The evolution of multi-drug resistance in food-producing animals as presented in Figure 16 is a worrisome situation. It demonstrates that in these animals an environment is created where multi resistant strains of all kind of species of microorganisms can survive and multiply. Examples of clinical relevance are ESBL-, and integron positive Enterobacteriaceae and MRSA, all organisms that are currently very commonly and increasingly present.

Figure 16. Trends in percentages of *E. coli* strains fully susceptible, resistant to one to a maximum of nine antimicrobial classes in broiler chickens, slaughter pigs and veal calves in The Netherlands from 1998 - 2007.

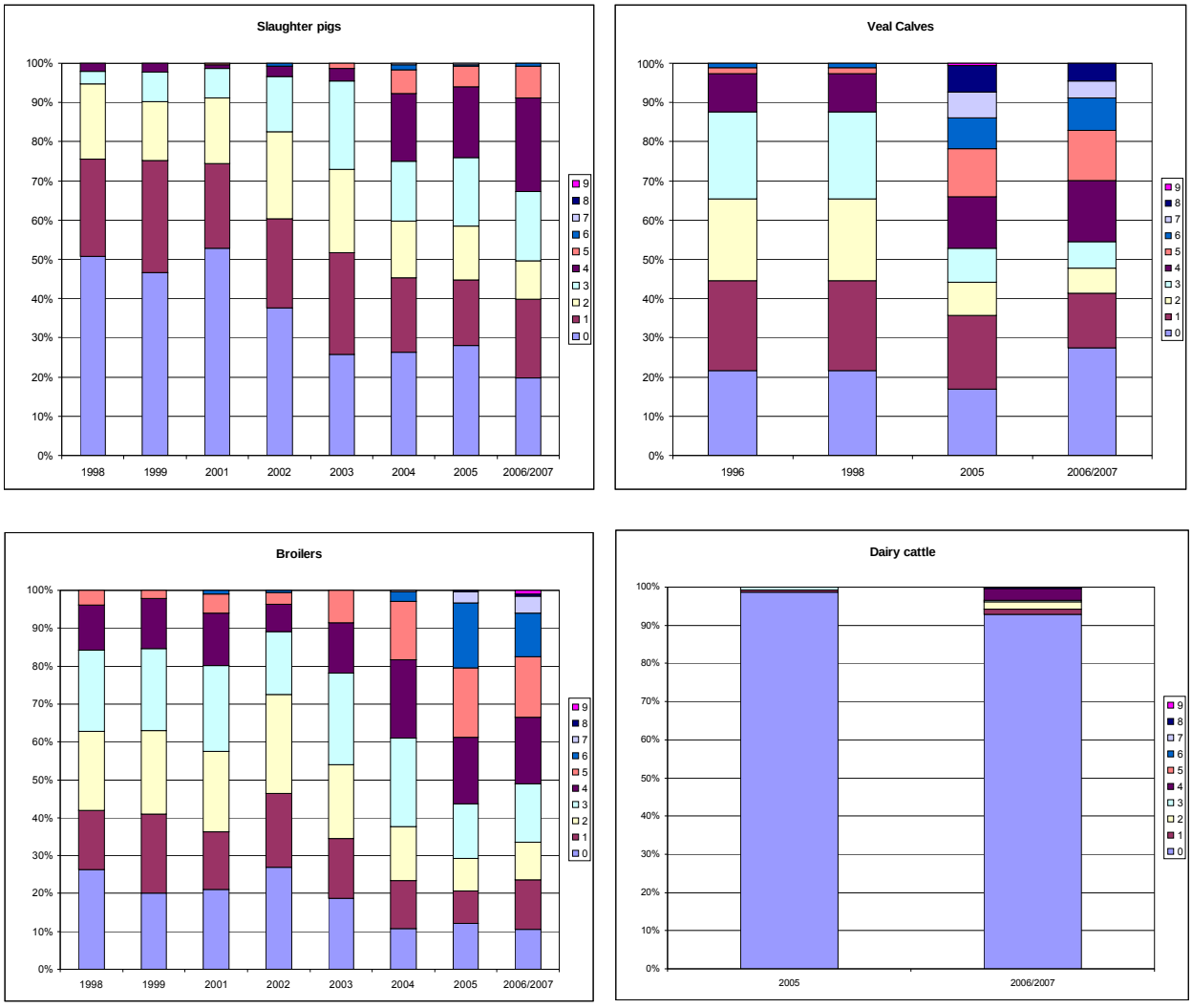
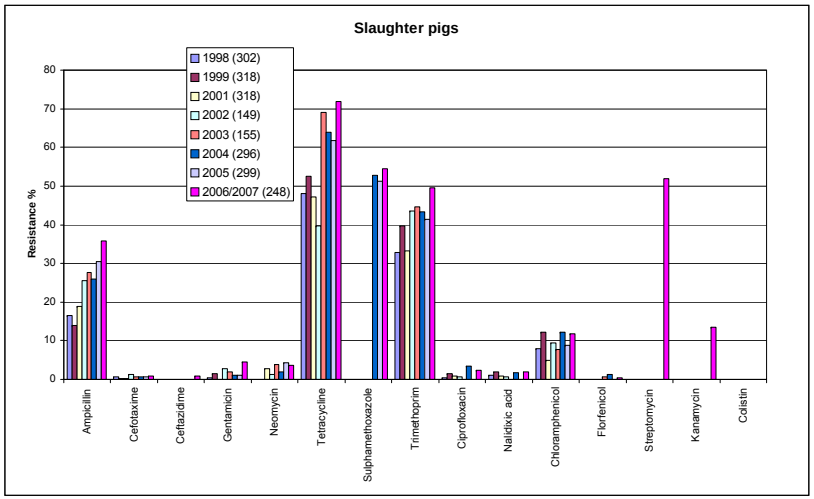
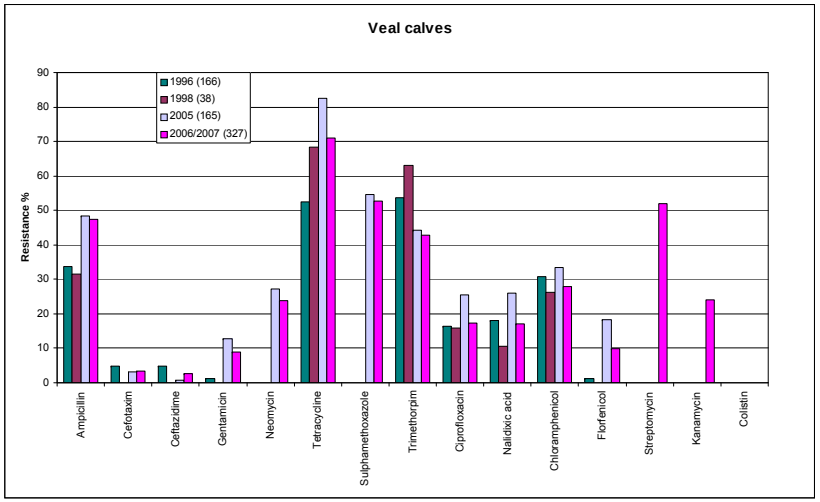
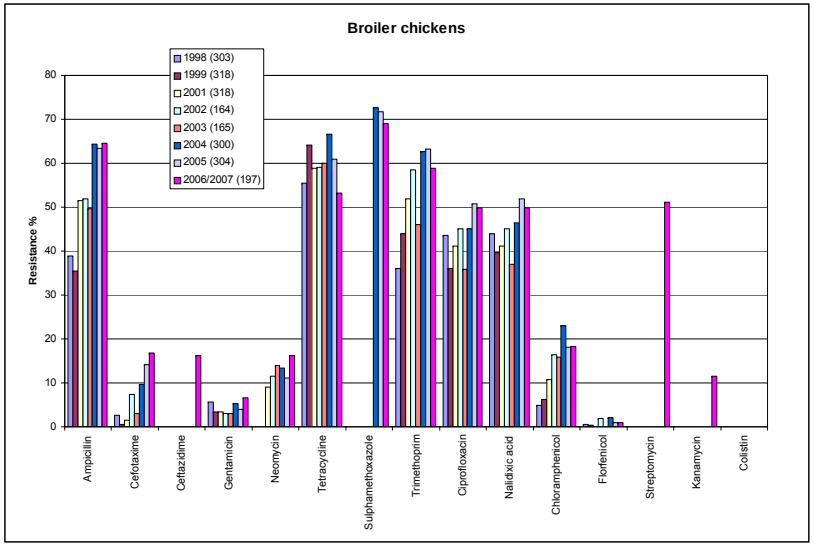
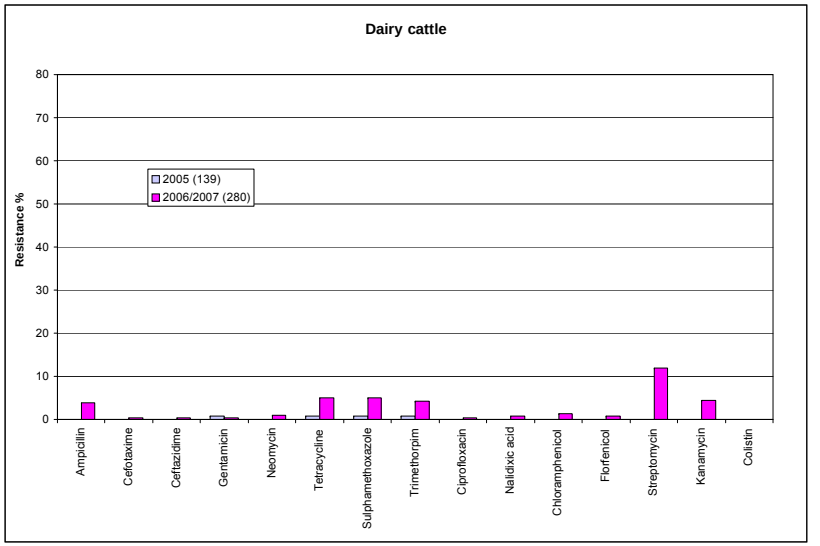


Figure 17. Trends in resistance (in%) of *E. coli* isolated from slaughter pigs and broilers in The Netherlands from 1998 - 2007









E. coli in raw meat products of food-animals

Table 22. Resistance (in %) of *E. coli* isolated from raw meat products at retail in the Netherlands in 2006/2007.

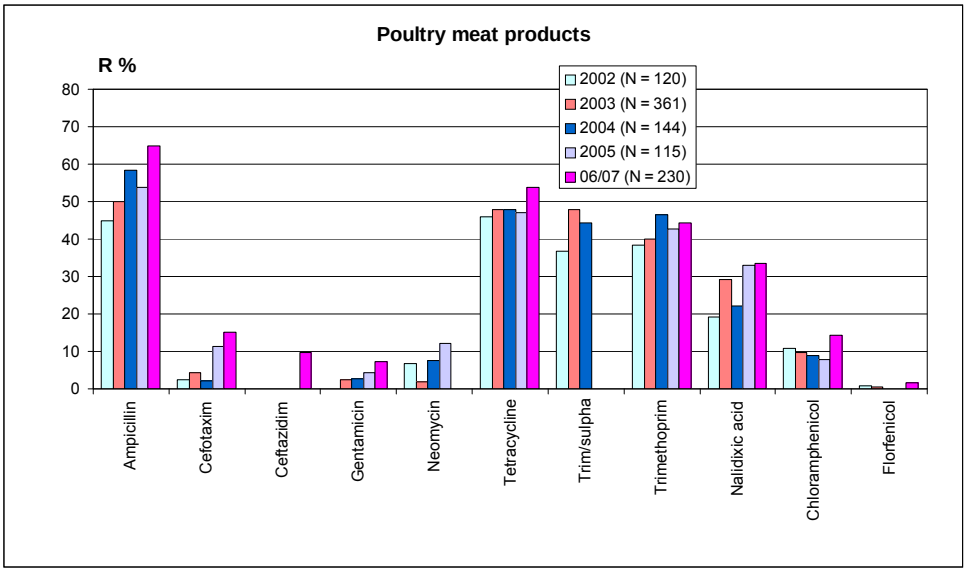
	Poultry meat	Biol poultry	Pork	Veal	Beef	Lamb
	N = 230	N = 31	N = 56	N = 16	N = 113	N = 10
Ampicllin	64.8	38.7	33.9	30.9	19.2	30
Cefotaxime	15.2	6.4	1.7	18.7	0.9	10
Ceftazidime	9.6	0	0	9.1	0	0
Gentamicin	7.4	3.2	1.7	6.3	0	10
Tetracycline	53.9	51.6	41.1	62.5	14.1	40
Sulphamethoxazole	66.1	35.4	48.2	68.8	45.1	70
Trimethoprim	44.3	32.3	26.8	43.8	7.9	30
Ciprofloxacin	33.9	12.9	3.6	12.5	3.6	10
Nalidixic acid	33.5	19.3	7.1	25.0	3.5	10
Chloramphenicol	14.3	12.9	10.7	12.5	7.1	0
Florfenicol	1.7	3.2	0	6.3	0	0
Streptomycin	33.9	21.4	24.3	27.3	10.8	37.5
Kanamycin	12.6	0	2.7	9.1	3.1	25
Colistin	1.3	0	0	0	0	0

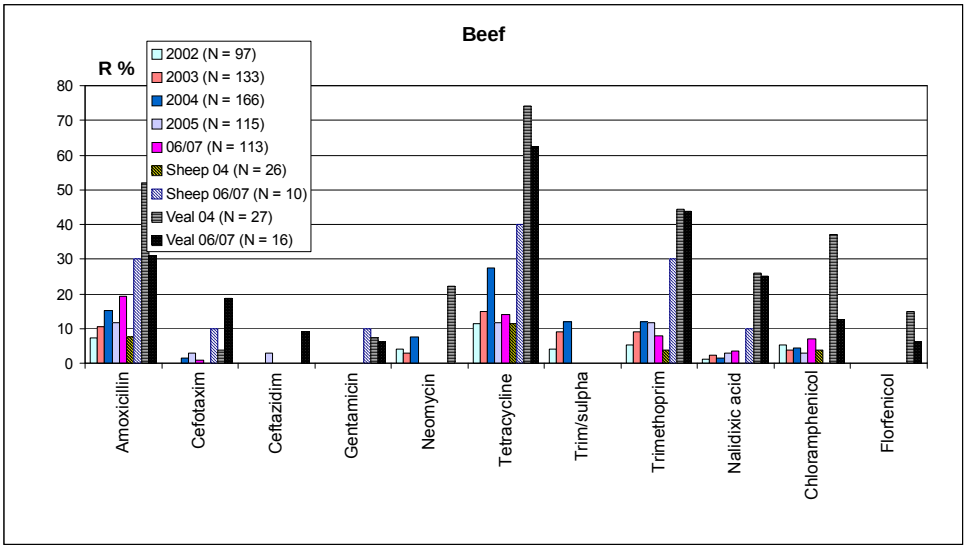
Resistance percentages of *E. coli* strains isolated from poultry products sampled at retail in the Netherlands were similar to those isolated as indicator organisms from faeces of Dutch broilers (Tables 21 and 22). However, like in 2005 resistance to the quinolones was substantially lower in poultry raw meat products. Resistance in isolates from biological poultry were always less resistant than those from conventional animals.

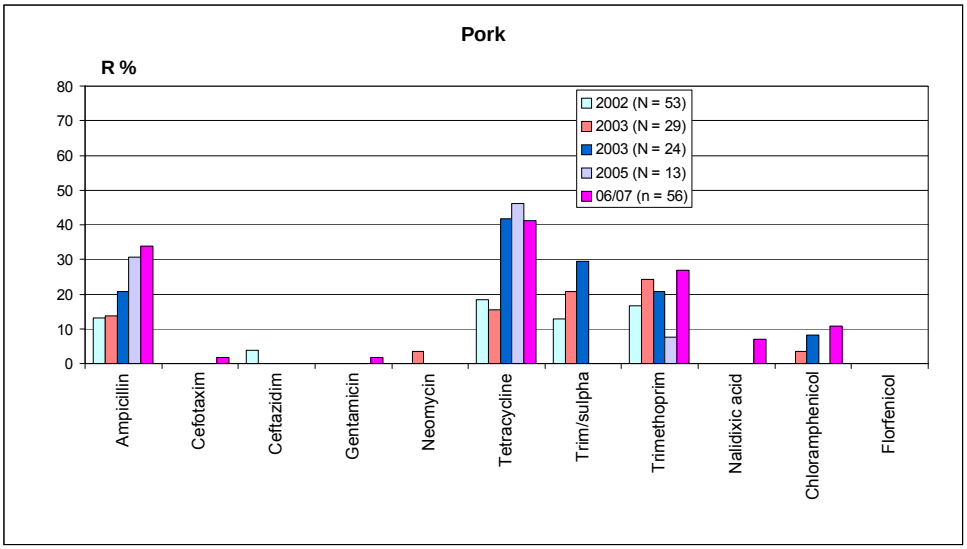
The numbers of strains isolated from veal are too small to conclude on differences with isolates from veal calf faeces. Resistance levels in isolates from beef are lower than those from veal (Table 22).

Figure 21 shows trends in resistances in the different meat products. The resistance percentages show a general tendency to increase, similar as observed in isolates from faeces

Figure 18. Trends in resistance (in%) of *E. coli* isolated from raw poutry meat products, beef, veal, sheep and pork, in The Netherlands from 1998 - 2007







Enterococcus faecium, Enterococcus faecalis

Highlights

Highest resistance levels were observed for tetracycline and erythromycin in both bacterial species. In broiler chickens the resistance levels show a tendency to decrease, while in the other animals the levels seem to be stable. Resistance to vancomycin remains present but is rare. Multi drug resistance is very common in veal calves, pigs and broilers, but not in dairy cows

The quantitative information on the MIC-distributions are summarized for all *E. faecalis* and *E. faecium* strains isolated from faecal samples from food-producing animals in 2006/2007 in Table 23. In Table 24 the calculated resistance percentages are presented for each bacterial species and specified by the different animal sources**.**

In table 23, if available, both the epidemiological cut-off values and clinical EUCAST breakpoints are presented. The MIC distributions show that using the cut-off values has no effect on the level of resistance percentages in the enterococci.

Highest resistance levels were observed for tetracycline and erythromycin in both species and flavomycin (intrinsic resistance in E. feacalis) and quinu/dalfopristin in *E. faecalis***.** (Tables 23 and 24, Fig. 22). The accuracy of the resistance percentages for quinu/dalfopristin are difficult to assess**.** The frequency distribution of the MICs for the streptogramins is complex and generally multimodal. The cut-off value for *E. faecium* has been defined by EUCAST at 1 mg/L, which seems very low and may overestimate the acquired resistance levels**.** For E. feacalis for quinu/dalfopristin no cut-off has been established yet. EFSA proposed to use 32 mg/L for this species, which seems appropriate.

Amoxicillin resistance was only observed at relatively low levels in *E. faecium* isolated from all animal species**.**

In 2006/2007 high level ciprofloxacin resistant *E. faecalis* isolates were observed (MIC $\geq$ 16 mg/L). These strains were isolated from veal calves and broilers, the animal species in which quinolones are predominantly used. In *E. faecium* also ciprofloxacin resistance occurred in these animal species, but the MICs were lower compared to *E. faecalis***.**

Table 23. MIC distributions (In %) for *E. faecalis* (N = 228) and *E. faecium* (N = 464) isolated in food producing animals in The Netherlands in 2006/2007.

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E. faecalis (N = 228)	MIC (%) distribution mg/L														R%
	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Amoxicillin			99.6	0.4											0.0
Linezolid			1.3	94.3	4.4										0.0
Tetracyline		21.9	8.8			0.4		9.2	25.0	34.6					69.3
Erythromycin			28.5	14.9	12.3	2.2	0.4	1.3	0.4	0.4	39.5				44.3
Vancomycin		3.5	61.0	35.1	0.4										0.0
Ciprofloxacin		19.7	64.5	13.6	0.4	0.4		0.9	0.4						1.8
Flavomycin					94.7	3.5	0.4		0.4				0.9		1.3
Salinomycin		3.5	64.0	18.0	10.5	3.5	0.4								3.9
Quinu/dalfopristin		0.9		1.3	3.5	34.2	54.8	4.8	0.4						0.4
Genta > 500										93.9	0.4		0.4	5.3	5.7
Strep > 2000												61.8	1.3	36.8	36.8
Chloramphenicol						67.5	18.0		11.0	3.5					14.5

E. faecium (N = 464)	MIC (%) distribution mg/L														R%
	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Amoxicillin			69.8	17.2	7.5	0.6	2.2	1.7	0.9						5.4
Linezolid			1.1	61.4	37.5										0.0
Tetracyline		46.8	1.1	0.2			0.2	1.3	12.5	37.9					51.9
Erythromycin			22.2	21.3	15.7	6.5	0.9	0.4			33.0	0.0			40.7
Vancomycin		71.8	16.8	10.6						0.9					0.9
Ciprofloxacin		7.3	28.4	29.1	29.1	5.8	0.2								6.0
Flavomycin					0.2					0.4	1.3	7.5	90.5		99.8
Salinomycin			23.5	61.4	2.2	11.9	1.1								12.9
Quinu/dalfopristin		20.5	10.3	26.5	36.6	4.1	1.1	0.9							69.2
Genta > 500										98.5	0.2		0.2	1.1	1.3
Strep > 2000												74.6	1.5	23.9	23.9
Chloramphenicol				0.2	1.3	76.3	15.7	5.8	0.6						0.6

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the epidemiological cut-off values used as breakpoints**.** The dashed bars indicate clinical breakpoints**.**

Table 24. Resistance percentages (%) of *E. faecalis* and *E. faecium* isolated from faeces from dairy cows, veal calves, slaughter pigs and broilers in The Netherlands in 2006/2007.

E. faecalis	**Dairy cows (35)**	**Veal calves (69)**	**Slaughter pigs (56)**	**Broiler chickens (68)**
Amoxicillin	0	0	0	0
Linezolid	0	0	0	0
Tetracyline	31.4	62.3	89.3	79.4
Erythromycin	11.4	47.8	51.8	51.5
Vancomycin	0	0	0	0
Ciprofloxacin	0	2.9	0	2.9
Bacitracin	8.6	21.7	14.3	23.5
Flavomycin	0	2.9	0	1.5
Salinomycin	0	0	0	13.2
Quinu/dalfopristin	0	1.4	0	0
Genta > 500	0	8.7	10.7	1.5
Strep > 2000	14.3	46.4	44.6	4.2
Chloramphenicol	5.7	31.9	16.1	0

E. faecium	**Dairy cows (123)**	**Veal calves (180)**	**Slaughter pigs (97)**	**Broiler chickens (49)**
Amoxicillin	0	11.7	3.1	2.0
Linezolid	0	0	0	0
Tetracycline	6.5	66.7	85.6	46.9
Erythromycin	12.2	61.7	34.0	49.0
Vancomycin	0	1.1	2.1	0
Ciprofloxacin	10.6	6.1	0	8.2
Bacitracin	43.1	34.4	17.5	63.3
Flavomycin	100	100	99	100
Salinomycin	0	1.7	30.9	46.9
Quinu/dalfopristin	54.5	70.6	88.7	63.3
Genta > 500	0	2.8	0	2.0
Strep > 2000	2.4	45.0	9.3	15.0
Chloramphenicol	0	1.7	0	0

Table 24 shows the resistance percentages for *E. faecalis* and *E. faecium* in isolates from different animal sources**.** Obviously resistance was more common in intensively reared food producing animals (veal calves, pigs and broilers) than in dairy cows reflecting the differences in husbandry and antibiotic use practices**.** Resistance to tetracycline and erythromycin is very common in these food producing animals**.** Salinomycin resistance is typically observed in pigs and broilers where ionophores are used as coccidiostatic agent in the feed.

Figure 19. Trends in resistance percentages of *E. faecium* and *E. faecalis* isolated from slaughter pigs, broilers and veal calves in The Netherlands from 1996 – 2007

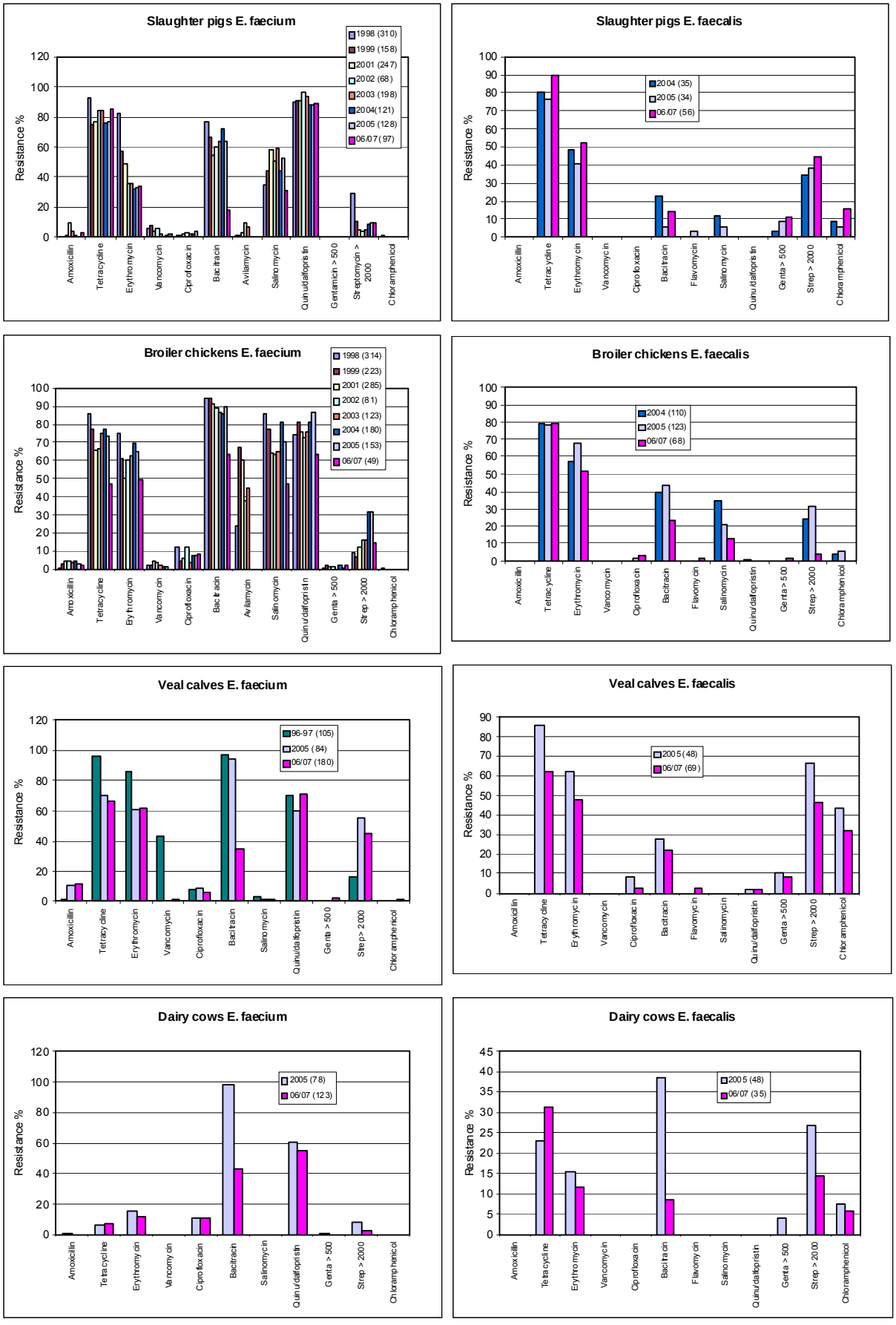
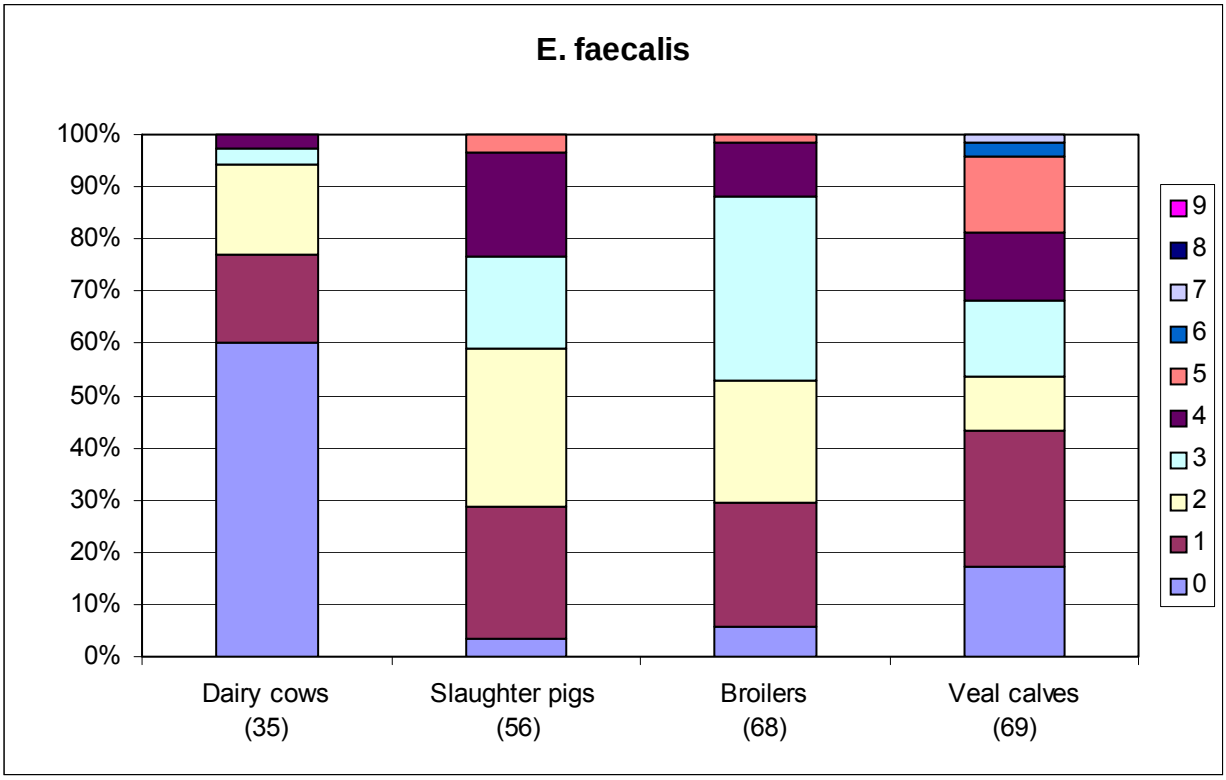


Figure 20. Percentages of *E .faecalis* and *E. faecium* strains fully susceptible, resistant to one to a maximum of nine antimicrobial classes in dairy cows, slaughter pigs, broiler chickens and veal calves in The Netherlands 2006 - 2007.



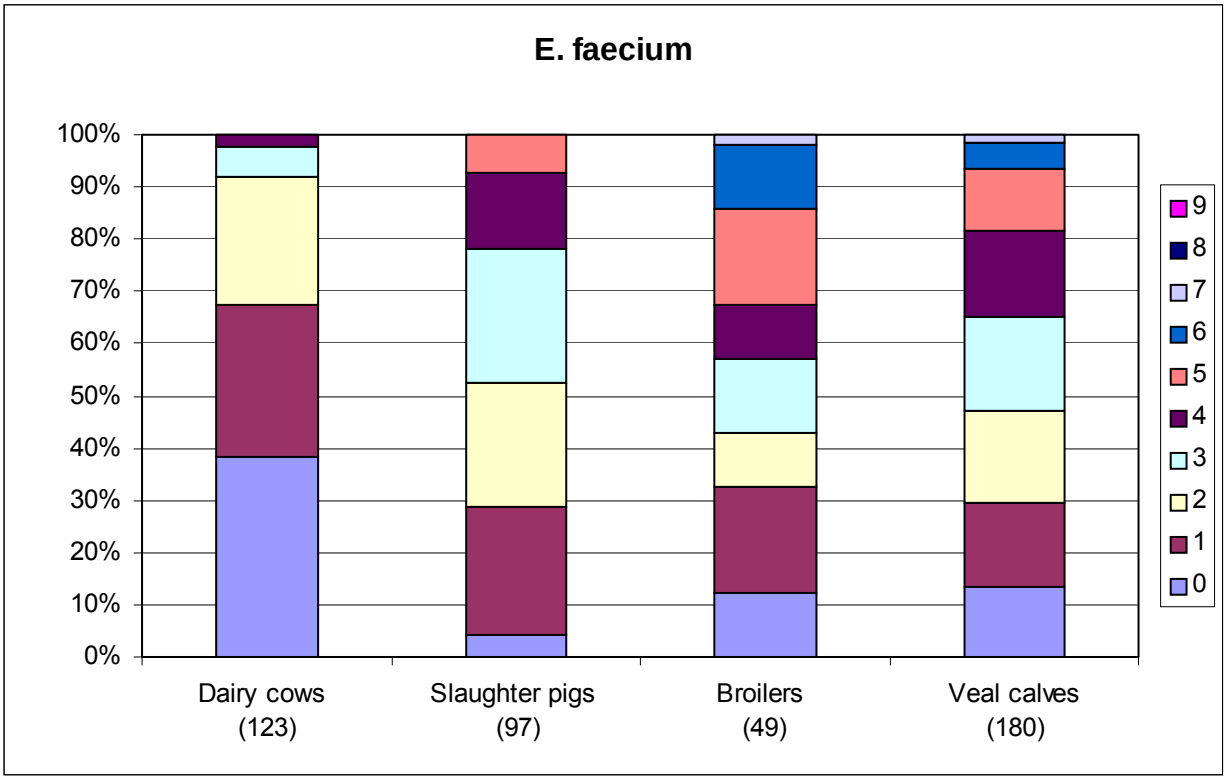


Figure 19 shows the variation in resistance levels over time. In broiler chickens the resistance levels show a tendency to decrease, while in the other animals the levels seem to be stable. Multi drug resistance is very common in veal calves, pigs and broilers, but not in dairy cows (Fig. 20).

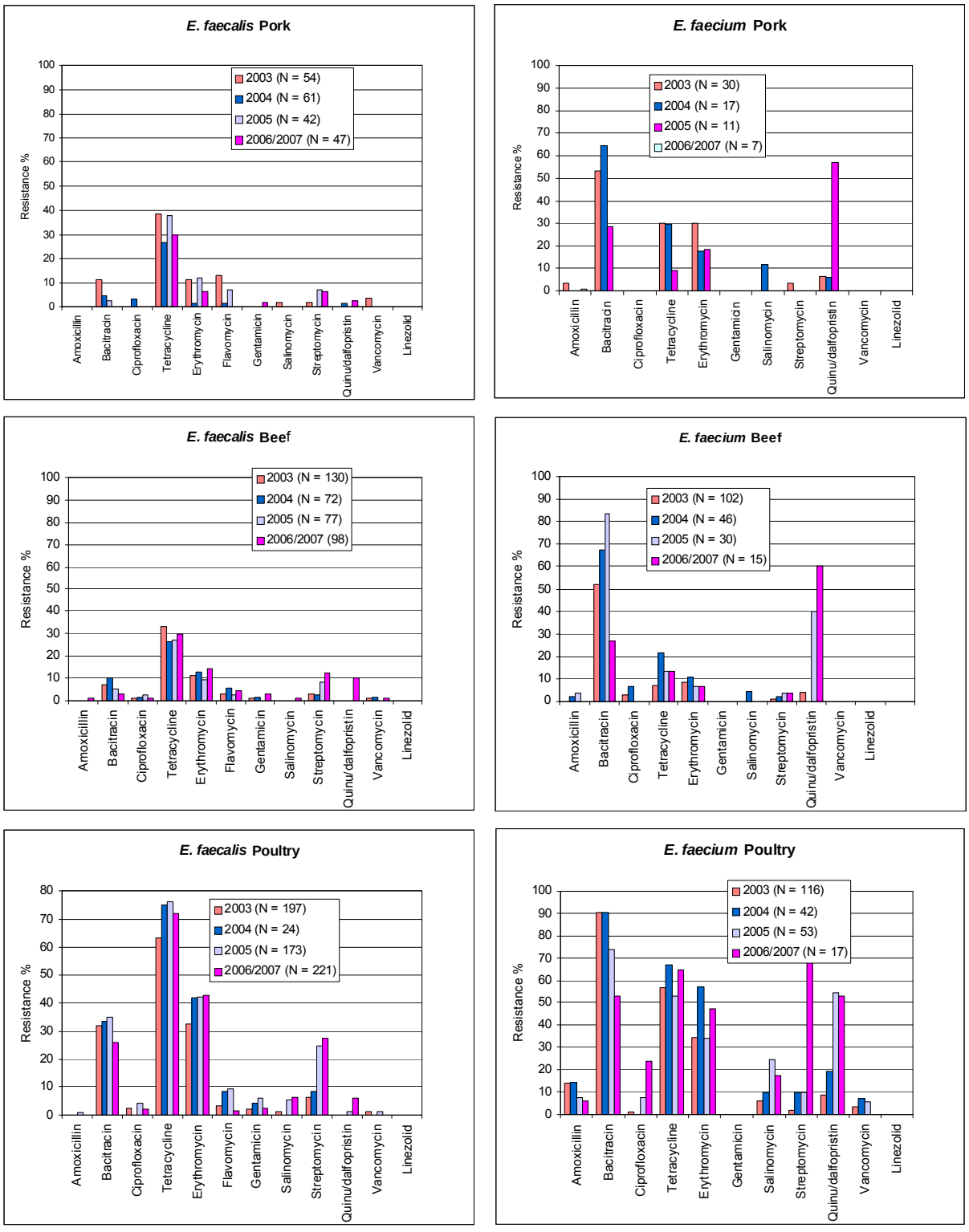
E. faecium and *E. faecalis* in raw meat products of food-animals

In comparison with isolation rates from faecal samples, *E. faecalis* was more frequently isolated than *E. faecium*, which may indicates that survival rates of these species on meat products are not identical. Except *E. faecalis* from broilers and beef, the numbers of isolates were too small to draw firm conclusions on both the occurrence and trends in resistance (Table 25, Fig. 24).
Nevertheless, resistance percentages in *E. faecalis* and *E. faecium* were very similar to those found in isolates from food animal faeces**.**
Vancomycin resistance was found in isolates from veal calves, slaughter pigs, poultry products and veal. One *E. faecalis* isolate from poultry raw meat was classified as vancomycin resistant (MIC 8 mg/L). This is a very rare finding and the presence of a vanA or B-gene needs to be confirmed.
Figure 24 shows the trend from 2003 to 2007 in isolates from raw meat products**.** Accurate trends cannot be observed and trend analysis is complicated by the relatively small numbers of strains per year. Obviously resistance is more common in *E. faecium* compared to *E. faecalis* except in poultry products**.**

Table 25. Resistance % of *E. faecalis* and *E. faecium* isolated from raw meat products from poultry, beef, pork, lamb and veal in the Netherlands in 2006/2007

E. faecalis	**Poultry N =173**	**Beef N = 77**	**Pork N = 42**	**Lamb N = 12**	**Veal N = 25**
Amoxicillin	0.6	0	0	0	4.0
Linezolid	0	0	0	0	0
Tetracycline	76.3	27.3	38.1	41.7	79.2
Erythromycin	42.2	9.1	11.9	8.3	32.0
Vancomycin	1.2	0	0	0	8.0
Ciprofloxacin	4.0	2.6	0	0	2.0
Flavophospholipol	9.2	2.6	7.1	8.3	16.0
Salinomycin	5.2	0	0	0	0
Quinu/dalfopristin	1.2	0	0	0	0
Genta > 500	5.8	0	0	8.3	8.0
Strep > 2000	24.9	8.1	7.1	25.0	36.0
Chloramphenicol	3.5	3.9	2.4	0	24.0
E. faecium	**Poultry N = 53**	**Beef N = 30**	**Pork N = 11**	**Lamb N = 4**	**Veal N = 9**
Amoxicillin	7.5	3.3	0	0	0
Linezolid	3.8	0	0	0	0
Tetracycline	52.8	13.3	9.1	0	44.4
Erythromycin	34.0	6.7	18.2	0	11.1
Vancomycin	5.7	0	0	0	0
Ciprofloxacin	7.5	0	0	0	0
Salinomycin	24.5	0	0	0	0
Quinu/dalfopristin	54.7	40.0	45.5	75.0	55.6
Genta > 500	0	0	0	0	0
Strep > 2000	9.6	3.3	0	0	0
Chloramphenicol	0	3.3	0	0	0

Figure 21. Trends in resistance percentages in *E. faecalis* and *E. faecium* isolated from raw meat products from poultry, beef and pork in The Netherlands from 2003 to 2007



Animal pathogens

Bovine respiratory disease pathogens *Pasteurella multocida* and *Mannheimia haemolytica*

In collaboration with the Animal Health Service in Deventer, the Netherlands, annually strains isolated from diagnostic specimens mostly taken at autopsy from cattle suffering from respiratory diseases, are tested for susceptibility by broth microdilution. This has been done since 1996. The number of strains isolated per year is limited, therefore every two years resistance data on respiratory disease pathogens from cattle are reported.
Although the resistance data may reflect a worst-case scenario of resistance in these pathogens, it still presents very important information on which resistance determinants occur and to what extend.

Highlights
Generally the resistance levels were higher in *M. haemolytica*. Tetracycline resistance occurred most frequently in both species, although substantially more frequently in *M. haemolytica*, in which species also resistance to amoxicillin, flumequine and chloramphenicol occurred frequently. Resistance to aminoglycosides was present in single isolates in *P. multocida* only. Single isolates resistant to tilmicosin and florfenicol were detected. For florfenicol this was the first time a resistant PMU is reported in The Netherlands.

In Table 26 the MIC distributions are presented for both *Pasteurella multocida* (PMU) and *Mannheimia haemolytica* (MHA). In Figure 22 the trends in resistance percentages are presented from 1996 to 2005.

The resistance profiles of PMU and MHA were not identical. Generally the resistance levels were higher in MHA. Tetracycline resistance occurred most frequently in both species, although substantially more frequently in MHA. In MHA also resistance to amoxicillin, flumequine and chloramphenicol occurred frequently. Resistance to aminoglycosides is present in single isolates in PMU only.
The resistance percentages for the quinolones are misleading. Based on the R breakpoint ≥ 2 mg/L, 0% PMU and 2.3% MHA were classified as resistant to enrofloxacin. However, for both genera substantial populations (app. 10% for PMU and 25% for MHA) show reduced susceptibility to enrofloxacin (MICs > 0.125 mg/L), demonstrating that acquired resistance to quinolones is commonly present.
Resistance to ceftiofur and tulathromycin was not detected. Single isolates resistant to tilmicosin and florfenicol were detected. For florfenicol this was the first time a resistant PMU is reported in The Netherlands.

Figure 22 shows that the resistance percentages vary substantially over the years. In PMU resistance shows a tendency to decrease, while in MHA the levels seem more stable.

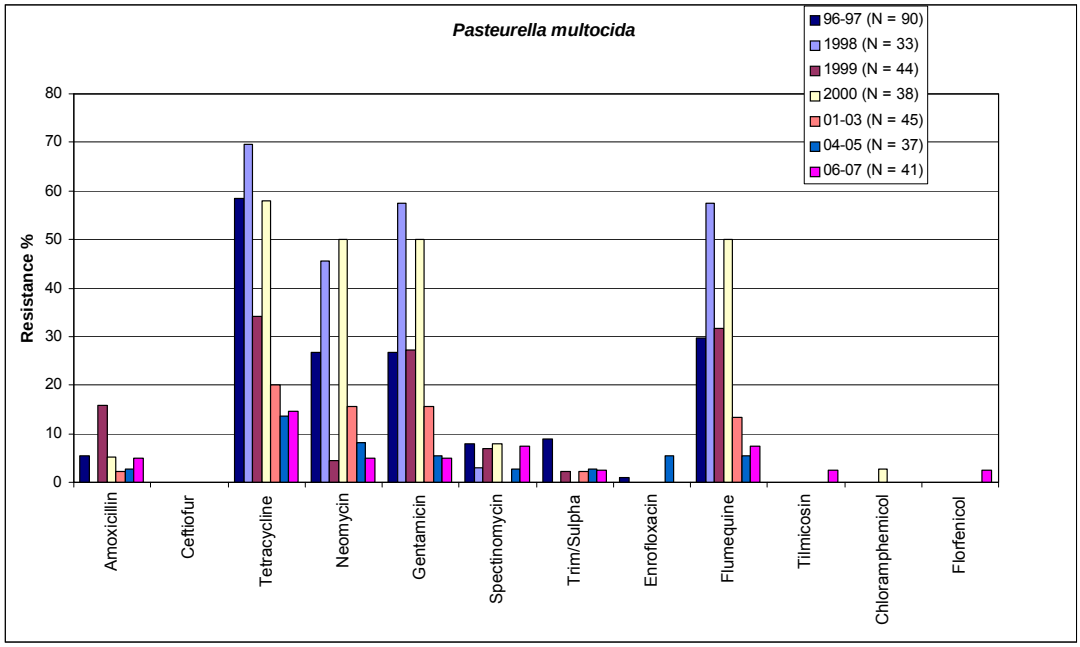
Table 26. MIC-distributions (in %) for bovine respiratory disease pathogens *P. multocida* and *M. haemolytica* isolate from Dutch cattle by the Animal Health Service in Deventer in 2006 and 2007.

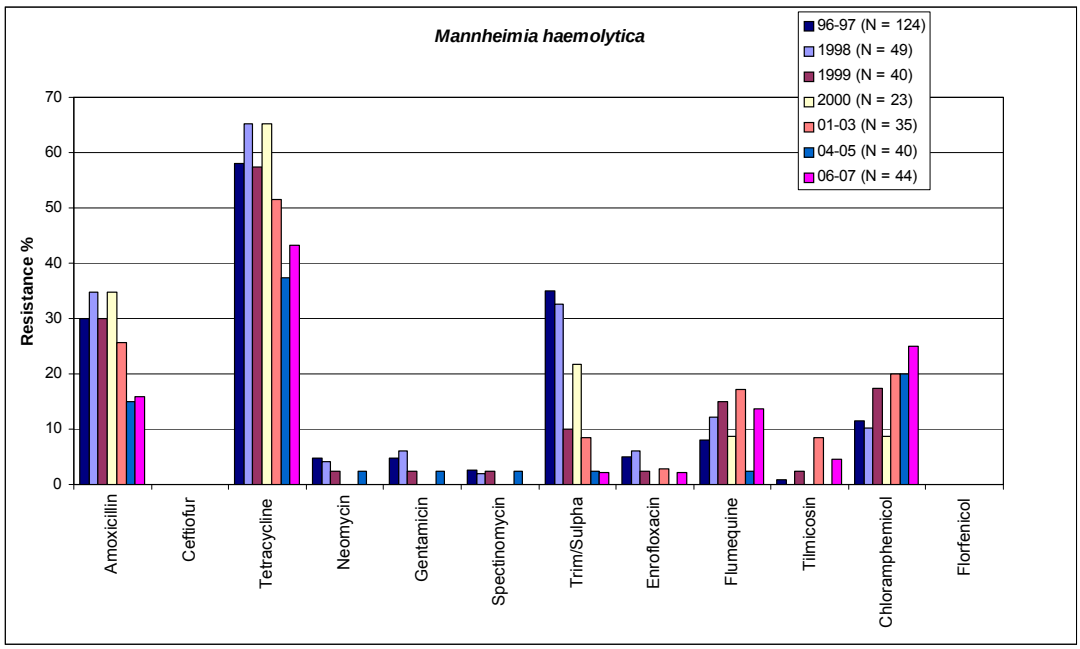
***P. multocida* (41)**	**MIC (%) distribution mg/L**															
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	R%
Amoxicillin					92.7		2.4						4.9			4.9
Ceftiofur			92.7	7.3												0.0
Tetracycline						43.9	9.8	22.0	2.4	7.3	4.9	4.9	4.9			14.6
Neomycin							12.2	36.6	24.4	19.5	2.4		2.4	2.4		4.9
Gentamicin						2.4	31.7	48.8	12.2					4.9		4.9
Spectinomycin										2.4	36.6	48.8	4.9		7.3	7.3
Trim/sulpha				80.5	4.9	4.9	4.9	2.4			2.4					2.4
Enrofloxacin		87.8	2.4		2.4	4.9	2.4									0.0
Flumequine					87.8			2.4	2.4		2.4	2.4	2.4			7.3
Tilmicosin						2.4	2.4	26.8	22.0	36.6	7.3	2.4				2.4
Tulathromycin							5.0	20.0	70.0	5.0						0.0
Chloramphenicol						33.3	47.6		4.8	4.8	9.5					0.0
Florfenicol					4.9	85.4	7.3					2.4				2.4

***M. haemolytica* (44)**	**MIC (%) distribution mg/L**															
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	R%
Amoxicillin					84.1								15.9			15.9
Ceftiofur			97.7	2.3												0.0
Tetracycline						27.3	27.3	2.3			18.2	20.5	4.5			43.2
Neomycin								2.3	52.3	45.5						0.0
Gentamicin						2.3	4.5	90.9	2.3							0.0
Spectinomycin											11.4	86.4	2.3			0.0
Trim/sulpha				75.0	2.3	4.5	15.9			2.3						2.3
Enrofloxacin		63.6	9.1	2.3	2.3	18.2	2.3			2.3						2.3
Flumequine					63.6	11.4			11.4	6.8	2.3	2.3	2.3			13.6
Tilmicosin						2.3		4.5	20.5	47.7	20.5	2.3	2.3			4.5
Tulathromycin							4.2	4.2	4.2	75.0	12.5					0.0
Chloramphenicol							10.0	65.0				20.0	5.0			25.0
Florfenicol						6.8	81.8	9.1	2.3							0.0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. The vertical bars indicate the breakpoints.

Figure 22. Trends in resistance (in %) of *P. multocida* and *M. haemolytica* isolated from 1996 – 2007 in the Netherlands.





Bovine mastitis pathogens *E. coli*, coliform bacteria, *S. aureus*, coagulase-negative staphylococci, *S. uberis* and *S. dysgalactiae*.

Highlights
The resistance levels for *E. coli* strains isolated from milk samples from cows suffering from mastitis were low except for ampicillin. In 2006/2007 the first ESBL-producing *E. coli*'s were isolated from mastitis.
The coliform bacteria showed a high level of resistance to ampicillin and to the combination with clavulanic acid. All isolates were susceptible to cefoperazone and cefquinome.
The *S. aureus* isolates tested were susceptible to most antibiotics. 9.2% were penicillin resistant. In 2006/2007 one *S. aureus* was identified to be MRSA. The strain belonged to the animal associated clonal complex 398. The coagulase negative staphylococci were more resistant than *S. aureus*. 56% were resistant to penicillin and 1.5% to oxacillin (mecA-positive).

Table 28. MIC-distributions (in %) for *E. coli* and coliform bacteria isolated from mastitis milk samples from Dutch cattle by the Animal Health Service in Deventer in 2006-2007.

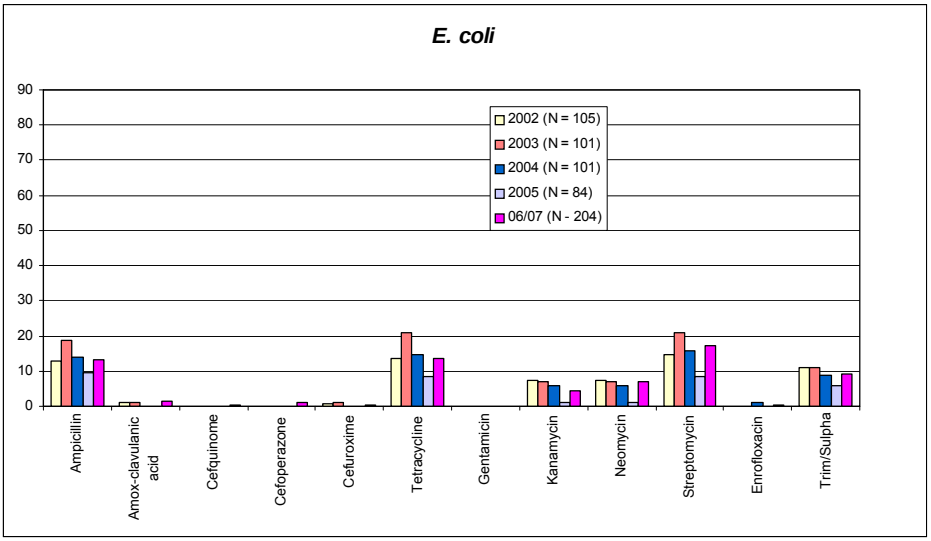
E. coli	MIC (%) distribution mg/L													
(204)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	R%
Ampicillin						6.9	33.3	39.7	6.9			13.2		13.2
Amox-clavulanic acid						2.9	31.9	48.5	12.7	2.5	1.0		0.5	1.5
Cefquinome		87.7	9.8	1.5				0.5		0.5				0.5
Cefoperazone		1.5	15.2	59.8	10.3	3.9	2.9	2.5		2.0	1.0	1.0		1.0
Cefuroxime							4.9	67.6	23.5	3.4	0.5			0.5
Tetracycline						12.7	53.4	19.1	1.0	0.5	1.5	11.8		13.7
Gentamicin				0.5	23.5	64.7	11.3							0.0
Kanamycin							4.4	65.7	19.6	2.9	2.9		4.4	4.4
Neomycin					0.5	34.3	50.0	7.8		0.5	6.9			6.9
Streptomycin								18.6	58.3	5.4	0.5	3.4	13.7	17.2
Enrofloxacin	83.3	14.7	0.5		1.0				0.5					0.5
Trim/Sulpha			86.8	2.9	1.0						9.3			9.3
Coliform	MIC (%) distribution mg/L													
(204)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	R%
Ampicillin						1.0	3.9	2.0	1.0	5.4	18.6	68.1		86.8
Amox-clavulanic acid						18.1	52.0	7.4	1.5	1.0	5.4	12.7	2.0	20.1
Cefquinome		81.9	14.7	2.5		0.5	0.5							0.0
Cefoperazone			7.4	45.1	16.2	6.9	12.7	7.4	2.9	1.5				0.0
Cefuroxime						3.9	48.0	22.5	8.8	8.3	8.3			8.3
Tetracycline					2.0	43.1	34.8	5.9	1.0	1.0	2.5	9.8		13.2
Gentamicin				11.8	72.5	15.2				0.5				0.5
Kanamycin						2.5	65.2	18.6	5.4	2.9	2.9	1.0	1.5	2.5
Neomycin					19.6	67.6	7.8	3.4		0.5	1.0			1.0
Streptomycin							16.7	58.3	12.7	2.5	2.9	1.5	5.4	6.9
Enrofloxacin	58.3	35.3	2.9	2.5	0.5	0.5								0.0
Trim/Sulpha			84.8	10.8	0.5		1.0				2.9			2.9

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. The vertical bars indicate the breakpoints.

In *E. coli* strains isolated from milk samples from cows suffering from mastitis the resistance levels were low to moderate. Only resistance to ampicillin, streptomycin, and tetracycline was present in percentages higher than 10%. One isolate was highly resistant to amoxicillin/clavulanic acid and to cefquinome indicating the presence of an ampC type Extended Spectrum Beta/Lactamase (ESBL) or a mutation in the promoter region of the chromosomal ampC-gene. A second isolate showed clear reduced susceptibility to cefquinome (MIC 4 mg/|L) indicating the presence of an ESBL as well. These isolates were also resistant to cefoperazone and cefuroxime. Resistance to the fluoroquinolone enrofloxacin is rare.
In coliform bacteria from mastitis cases (Klebsiella, Enterobacter), resistance to ampicillin was very common based on chromosomal beta-lactamases. Also resistance to amoxicillin-clavulanic acid is so often present that empiric treatment with this combination should be discouraged in cases of severe and acute mastitis without a culture result.
All isolates were susceptible to the third generation cephalosporins, cefoperazone and cefquinome and the fluoroquinolone, enrofloxacin.

Fig. 23 demonstrates that both in *E. coli* and in coliform mastitis pathogens the resistance levels remain stable over the years.

Figure 23. Trends in resistance percentages for *E. coli* and coliform bacteria isolated from clinical mastitis cases in dairy cattle in the Netherlands from 2002 – 2007.



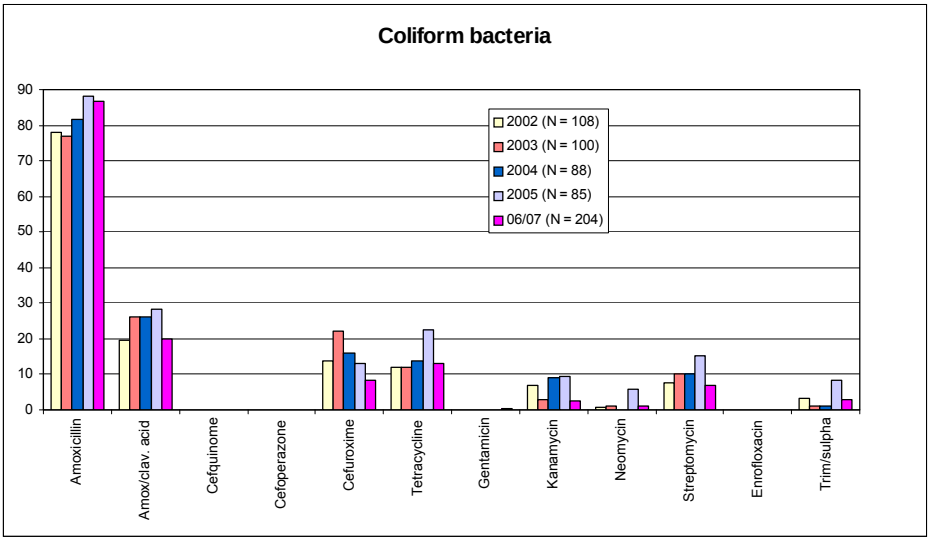


Table 29. MIC-distributions (in %) of *S. aureus* and coagulase-negative staphylococci (CNS) isolated from clinical mastitis cases in dairy cattle by the Animal Health Service in Deventer in 2006/2007.

S. aureus (196)	MIC (%) distribution mg/L												R%
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	
Penicillin	89.8	1.0				5.6	1.0	1.0	1.5				9.2
Oxacillin	1.5	16.8	39.8	39.8	1.0	0.5		0.5					0.5#
Amox-clavulanic acid		44.9	40.3	8.2	5.6	0.5	0.5						0.5#
Cephalothin	1.0	26.0	58.7	12.8	0.5		0.5	0.5					0.5#
Tetracycline			11.7	80.1	5.1					3.1			3.1
Kanamycin				0.5	1.5	24.0	56.1	17.9					0.0
Neomycin		1.0	5.6	50.0	40.3	3.1							0.0
Streptomycin					1.0		14.8	61.2	19.4	0.5		3.1	3.6
Erythromycin			25.0	69.4	4.1					1.5			1.5
Clindamycin	2.1	32.3	61.5	2.1	1.0				1.0				1.0
Pirlimycin			3.6	36.4	47.7	8.7	2.1	0.5		1.0			3.6
Trim/sulpha		96.9	3.1										0.0
CNS (199)	MIC (%) distribution mg/L												R%
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	
Penicillin	64.3	2.5	3.5	7.5	9.0	2.5	4.0	1.0	5.5				56*
Oxacillin	9.5	29.6	35.2	16.1	6.5	1.5			1.5				1.5#
Amox-clavulanic acid		55.3	28.6	10.6	4.5		0.5			0.5			1.5#
Cephalothin	3.0	31.3	46.0	14.1	4.5	0.5	0.5						1.5#
Tetracycline		3.0	46.7	35.2	1.5	2.5			0.5	10.6			11.1
Kanamycin				20.6	34.7	30.2	10.1	2.5	0.5		0.5	1.0	1.5
Neomycin		49.7	24.1	16.6	5.5	2.0	0.5	0.0	1.0	0.5			0.5
Streptomycin				2.5	7.5	24.6	37.2	17.6	3.5	0.5	2.0	4.5	7.0
Erythromycin		2.0	39.2	50.8	3.0		1.0		1.5	2.5			4.0
Clindamycin	5.0	33.2	42.7	10.1	5.5	1.5	0.5	1.0	0.5				2.0
Pirlimycin		2.0	23.6	40.7	16.6	4.0	6.0	5.5	1.0	0.5			13.1
Trim/sulpha		71.9	12.6	13.1		0.5		1.0	1.0				2.0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. The vertical bars indicate the breakpoints.
* All isolates with penicillin MIC ≤ 0.06 mg/L positive for penicillinase production were classified resistant to penicillin.
# All isolates with oxacillin MIC > 4 mg/L were mecA-positive. These isolates were also classified R for amox-clavulanic acid and cephalothin.

In spite of the intensive use of antibiotics in the control of bovine mastitis in the Netherlands, the *S. aureus* isolates tested were susceptible to most antibiotics. In 2006/007 9.2% of the isolates were penicillinase producers and one oxacillin-resistant isolate was detected. This isolate was confirmed to be MRSA by molecular techniques and belonged to the currently widespread occurring clonal complex 389, which are very common in pigs and veal calves in The Netherlands. It remained a single observation indicating that in dairy cattle this MRSA-clone is rare.

The coagulase negative staphylococci were more resistant than *S. aureus.* Penicillin resistance based on MICs and cefinase test was similar as in previous years. Three oxacillin resistant mecA-positive isolates were found that were also classified resistant to amoxicillin-clavulanic acid and cephalothin.

Resistance to pirlimycin was commonly presents (13.1%) and substantially higher that the related but more potent clindamycin (2%).

Although the numbers of strains included were relative large, the trends in resistance in fig. 24 may be affected by selection bias and not reflect true trends. Resistance to the lincosamides seems to have decreased after 2004. However in that year we changed from using lincomycin or clindamycin in our test panel, which will have affected the results.

Figure 24. Trends in resistance percentages for *S. aureus* and coagulase negative staphylococci isolated from mastitis milk in the Netherlands from 2002 - 2007.

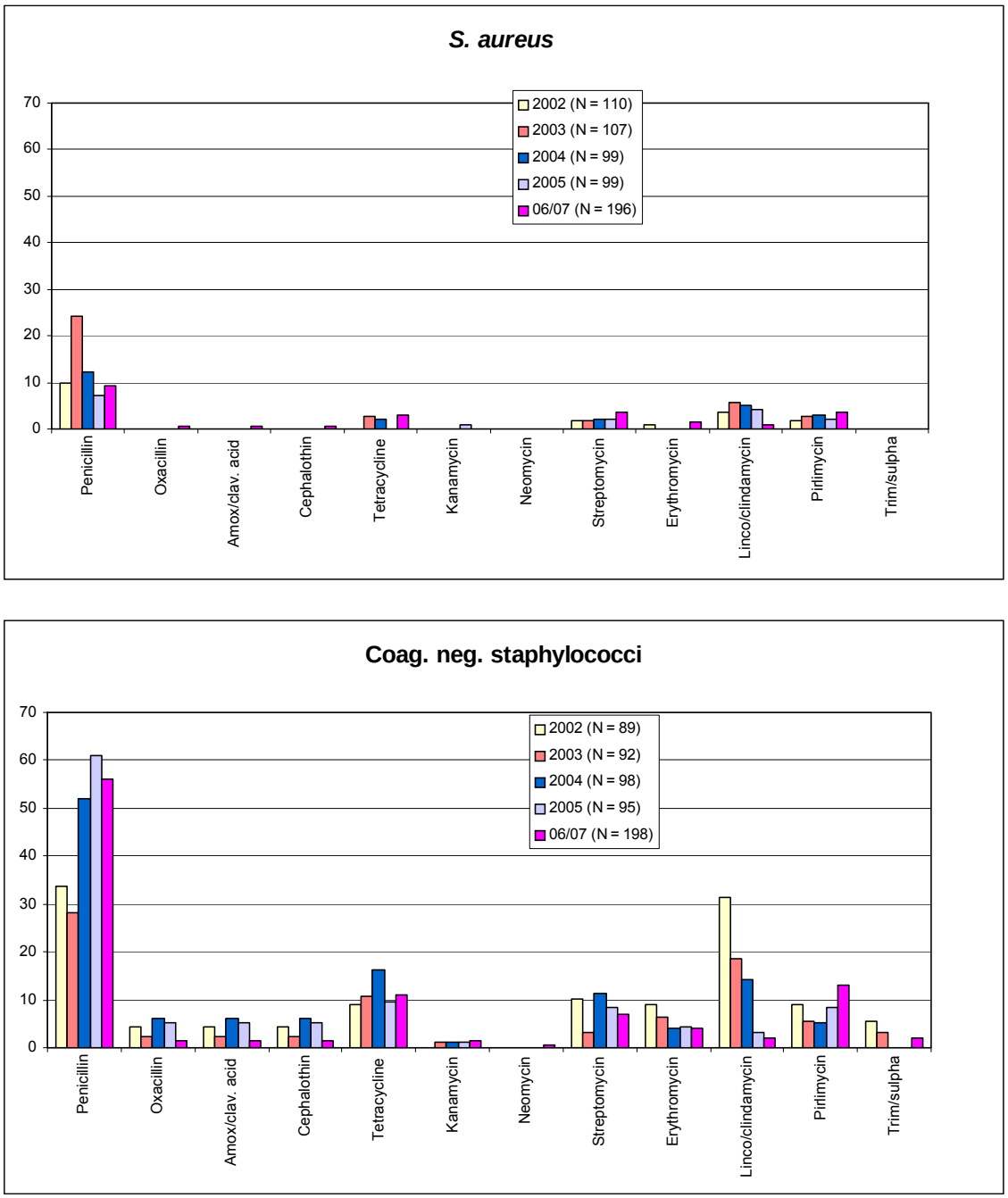


Table 30. MIC-distributions (in %) of *S. uberis* and *S. dysgalactiae* isolated from mastitis milk samples from Dutch cattle by the Animal Health Service in 2006/2007.

S. uberis	**MIC % distribution (µg/ml)**															
(202)	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	R%
Penicillin	27.7	9.4	3.0	13.9	33.7	5.4	0.5	5.0	1.5							1.5
Cephalothin			0.5	11.4	24.3	7.4	34.7	13.9	1.5	0.5		5.0	1.0			5.9
Erythromycin	2.5	32.2	39.6	1.5		1.0	5.4	2.5	1.0	0.5			13.9			23.3
Clindamycin		11.4	36.1	6.9	0.5	0.5	0.5	1.5	22.3	3.5	2.5	2.5	11.9			42.6
Lincomycin			2.0	27.2	1.0	1.5	4.0	16.8	2.5	1.0	2.0	2.0	40.1			45.0
Pirlimycin	0.5	3.5	46.0	3.5	2.0		1.0	9.4	14.4	6.9	0.5	1.5	10.9			34.2
Trim/sulpha		1.0	6.9	55.9	27.7	5.9	0.5	0.5					1.5			1.5
Tetracycline				0.5	19.8	35.6	2.5					2.0	26.7	7.4	5.4	41.6

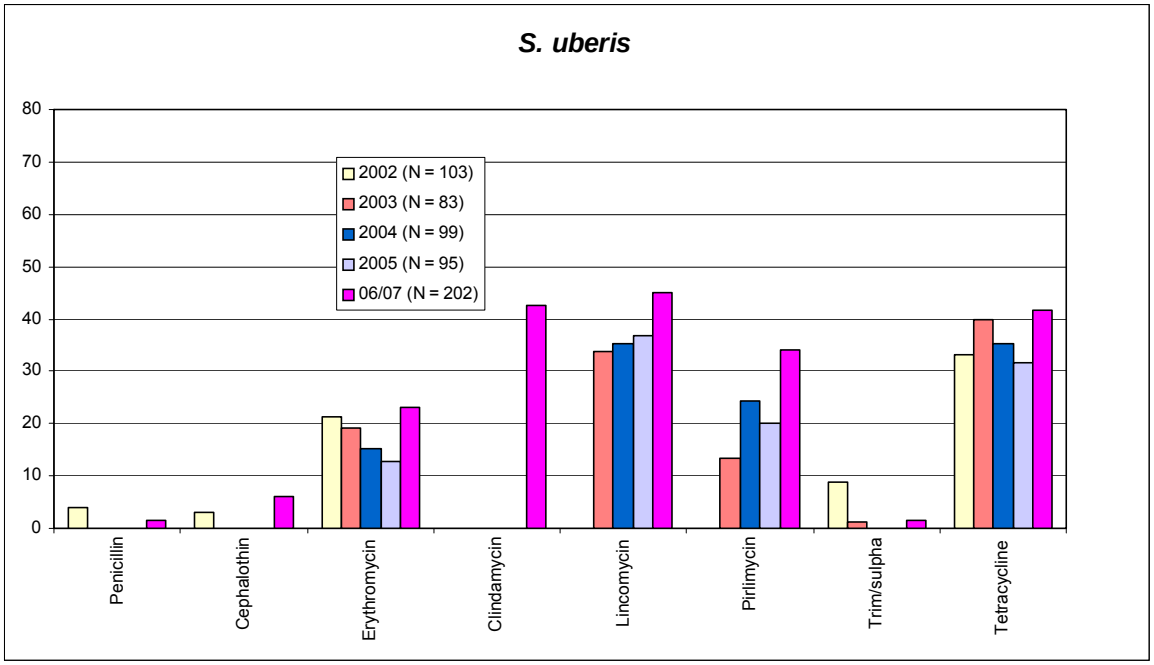
S. dysgalactiae	**MIC % distribution (µg/ml)**															
(201)	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	R%
Penicillin	98.0	1.0	0.5	0.5												0.0
Cephalothin				57.7	41.3	1.0										0.0
Erythromycin	2.0	42.8	47.3						0.5		1.0		6.5			8.0
Clindamycin	0.5	1.0	58.2	25.4	1.0			6.5	1.5	0.5			5.5			7.5
Lincomycin				39.8	23.9			3.0	10.4	7.5	1.5	0.5	13.4			22.9
Pirlimycin	0.5	1.5	52.7	28.4	3.0		0.5	2.0	3.5	2.5	0.5	0.5	4.5			11.4
Trim/sulpha		2.5	46.8	48.8	1.5	0.5										0.0
Tetracycline						0.5	0.5	1.0	9.0	14.9	2.5	1.0	18.9	48.8	3.0	74.1

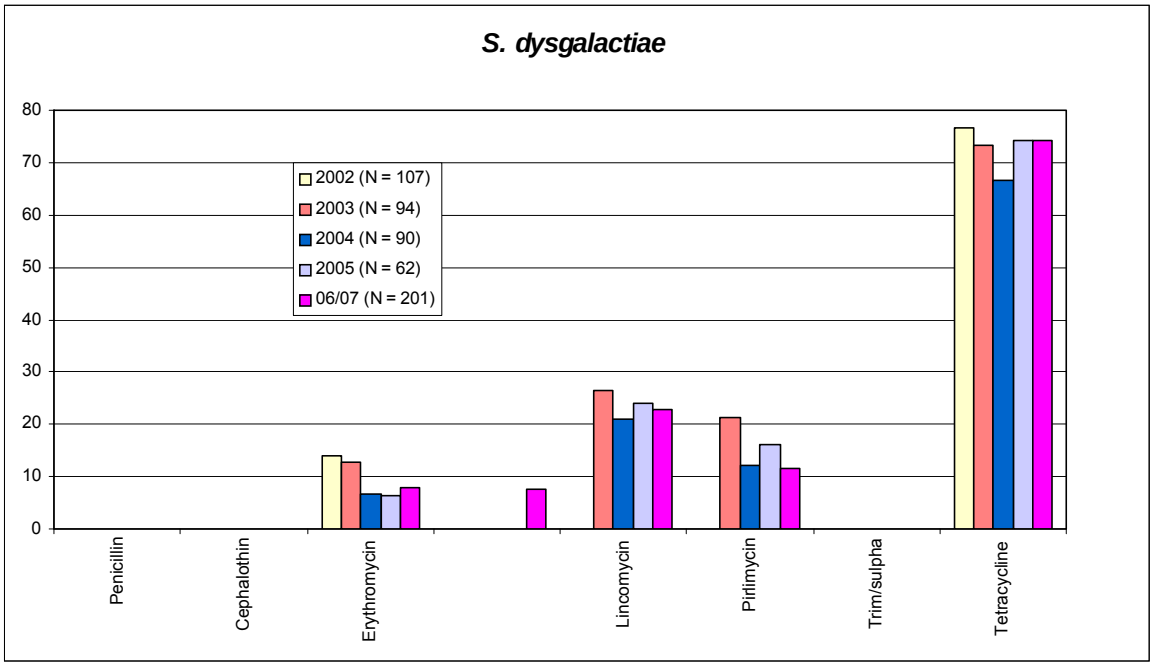
The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. The vertical bars indicate the breakpoints.

In 2006/2007 almost all isolates of *S. uberis* and all *S. dysgalactiae* were susceptible to penicillin and therefore also to all other beta-lactams (Table 30). The *S. uberis* isolates resistant to cephalothin were most likely misidentified enterococci. *S. uberis* shows a bimodal distribution of the penicillin MICs indicating that subpopulation of strains with MICs ≥ 0.125 mg/L with acquired resistance occurred. This indicates the presence of altered penicillin binding proteins as resistance mechanism as described for *S. pneumoniae.*
Resistance to erythromycin and the lincosamides (lincomycin and pirlimycin) occurred most frequently in *S. uberis.* Resistance to tetracycline was most common in *S. dysgalactiae*.
In 2006/2007 for the first year clindamycin was included in the tests. The fact that the resistance levels were not identical to those for linco- and pirlimycin was most likely related to inadequate breakpoints for the drugs, underestimating the resistant populations for all three lincosamides.

Resistance to erythromycin and the lincosamides show an increase in 2006/2007 in *S. uberis.*
Resistance to the other antibiotics was stable.

Figure 25. Trends in resistance percentages for *S. uberis* and *S. dysgalactiae* isolated from mastitis milk in The Netherlands from 2002 - 2005.





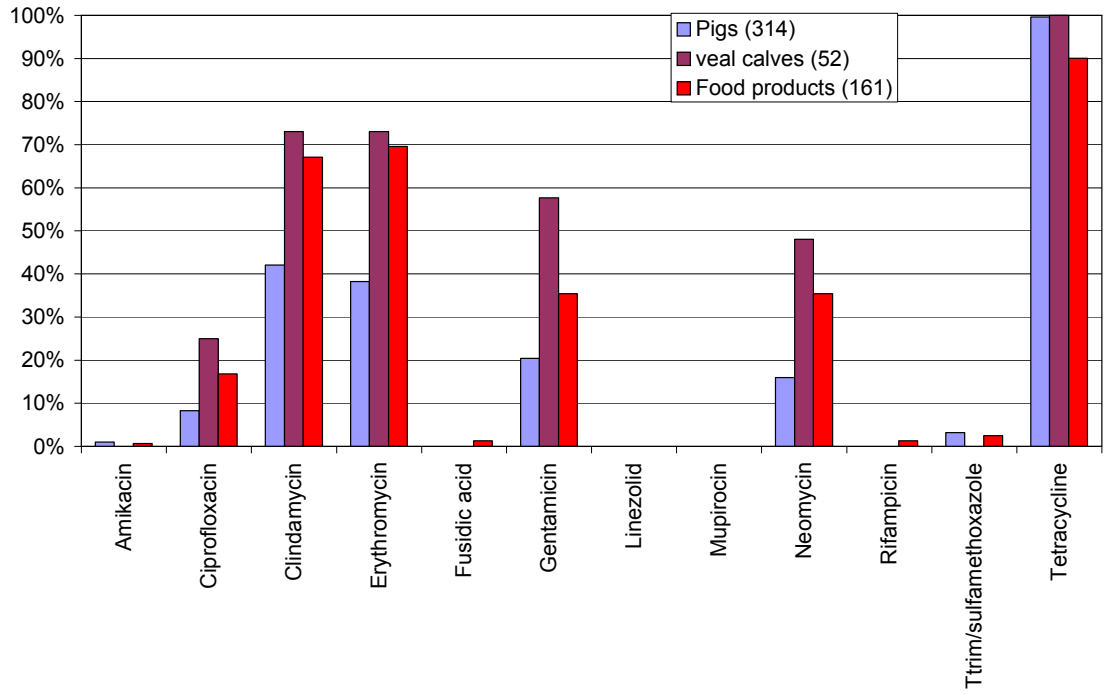
III Appendices

Appendix I. Resistance determinants in MRSA strains isolated in 2006/20077 from pigs, veal calves, and food products in The Netherlands[17]

After the discovery of the high prevalence of MRSA in pig and later also in veal calf farming and in persons at risks like veterinarians and farmers, a large research program started in The Netherlands. As part of this research program intensive prevalence studies were conducted in different animal species and all isolates were sent to the National Institute for Public Health and the Environment (RIVM-Bilthoven) for molecular typing and to the Central Veterinary Institute (CVI-Lelystad) for susceptibility testing. The purpose of these studies were to identify the genetic characteristics of these MRSA's and to determine if next to the *mecA*-gene cluster additional resistance genes would be acquired as a result of different antibiotic use practices in food producing animals.
The present study describes the Minimum Inhibitory Concentrations (MICs) of MRSA's isolated from pigs, veal calves and animal food products in the Netherlands in 2007. Moreover it describes the resistance associated genes present is a subset of 60 of the MRSA's.

In 2007 508 MRSA strains were sent to CVI-Lelystad for susceptibility testing. These strains were isolated from pigs (piglets, sows and slaughter pigs, N = 295), from veal calves (N = 52) and from food products (N = 161). Susceptibility was tested by broth microdilution according to CLSI guidelines using a custom format Sensititre panel.
Sixty-four isolates from pigs (N = 22), veal calves (N = 21) and food products (N = 22) were genetically characterized using the MRSA micro-array of Clondiag Chip Technologies. The isolates were also tested for vancomycin susceptibility using screen plates according to CLSI M31-A2.

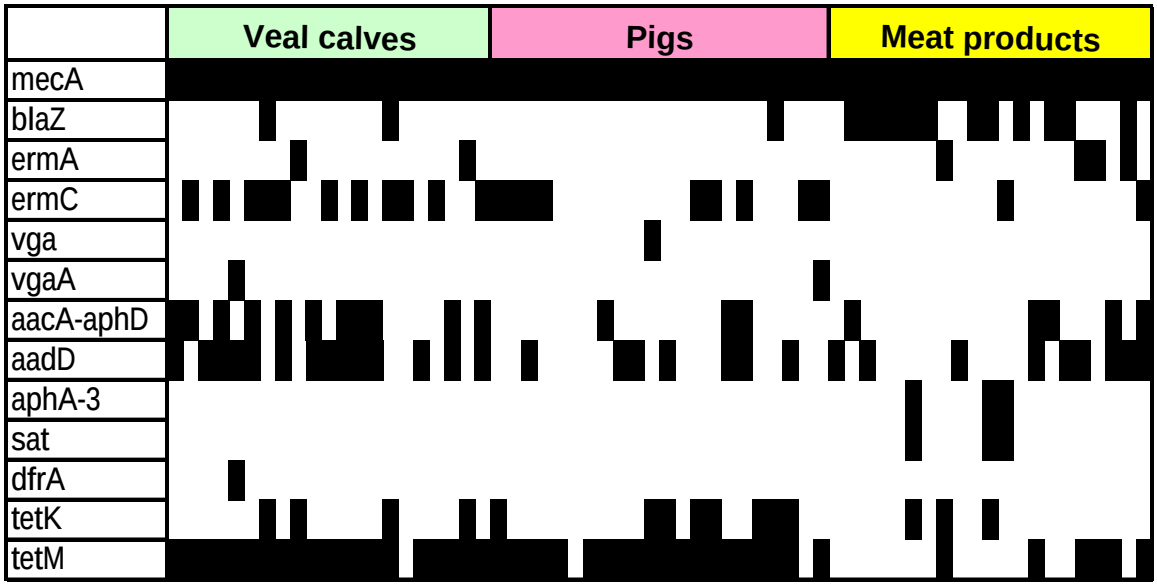
Figure 26. Resistance percentages of 527 MRSA's isolated from pigs (N = 314), veal calves (N = 52) and meat products (N = 161) isolated in the Netherlands in 2006 and 2007.



[17] Dik Mevius, Cindy Dierikx, Denice Verheijen, Kees Veldman, Ben Wit, Peter van der Wolf, Haitske Graveland, Xander Huijsdens, Arjen van der Giessen. On behalf of the Dutch working group SOM. Resistance and virulence determinants in MRSA strains isolated in 2007 from pigs, veal calves, and food products in The Netherlands. ASM Conference on Antimicrobial Resistance in Bacteria of Animal origin, June 2008, Copenhagen

All isolates from pigs and veal calves belonged to CC398, while the isolates from meat products were more variable. Next to methicillin resistance, almost all isolates were resistant to tetracycline. This occurred predominantly in isolates from meat products not belonging to CC398. Resistance to clindamycin, erythromycin, gentamicin, neomycin and ciprofloxacin was observed in frequencies varying from 10 - 70% of the isolates. Resistance genes detected were *mecA, tetM, aadD*, *ermC, aacA-aphD, tetK, blaZ, vgaA, sat, aphA3, vga* in order of most frequent occurrence.
It can be concluded that animal associated MRSA harbours a variety of additional resistance determinants.

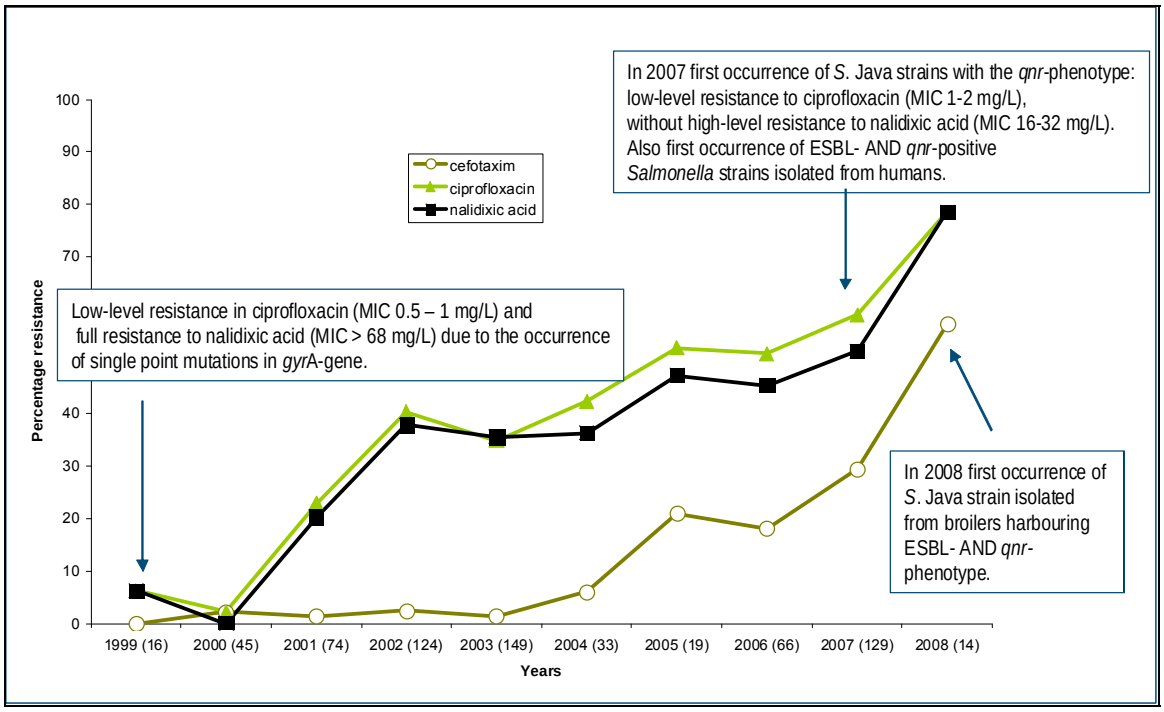
Figure 27. Resistance genes detected by MRSA array in 62 MRSA isolates from veal calves, pigs and meat products in The Netherlands in 2006 and 2007.



Appendix II. ESBLs and qnr-genes in *Salmonella* Paratyphi B var Java isolated from Dutch poultry[18]

Salmonella Paratyphi B var. Java is the most predominant serovar isolated from broiler chickens in The Netherlands since approximately 1998. The clone harbours a 2300 bp class 2 integron in the chromosome with *dfrA1, sat1*, *aadA1/C* gene cassettes. *S.* Java is well adapted to poultry colonizing chickens and the housing facilities very readily, but is not specifically pathogenic and rarely causes food borne infections in humans. As a result of the use of quinolones in poultry an increase in low-level resistance to ciprofloxacin and full resistance to nalidixic acid was observed starting in 1999 (fig 28). This was caused by single point mutations in the *gyrA* gene. Since 2003 a rapid increase in cefotaxime resistance was observed in both *E. coli* and in *S.* Java from Dutch broilers (fig 28). This is indicative of the presence of extended spectrum *beta*-lactamases (ESBLs) in both bacterial species and of horizontal transmission of the genes in poultry. Molecular characterization of cefotaxime resistant isolates of *S*. Java isolates from 2006 showed that different ESBL genes were present (fig 29) and most of them were also found in *E. coli* This makes horizontal transmission of these genes even more likely to exist. The *beta*-lactamases deteced in *S*. java were $bla_{\text{TEM-20, 52}}$, $bla_{\text{CTX-M-1, 2}}$

Figure 28. Dynamics in resistance percentages of ciprofloxacin, nalidixic acid and cefotaxime for *S* Java isolates from Dutch poultry from 1999 – 2008.

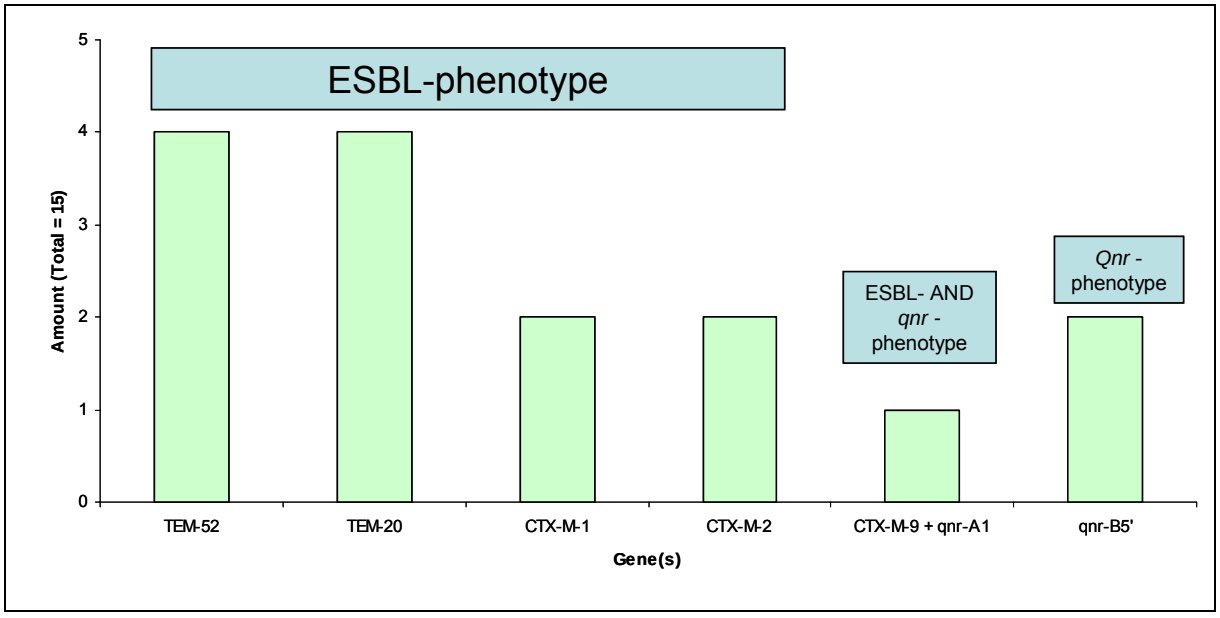


[18] Cindy Dierikx, Kees Veldman, Muna Anjum, Muriel Mafura, Marga Japing, Ruud Baaiman , Wilfrid van Pelt, Henny Maas and Dik Mevius. Extended Spectrum ß-lactamases and qnr-genes in Salmonella Paratyphi B var Java isolated from Dutch Poultry. ASM Conference on Antimicrobial Resistance in zoonotic bacteria and food-borne pathogens, 15 – 18 June 2008, Copenhagen, Abstract B89, page 64 - 65.

At the end of 2007 and the beginning of 2008 *S.* Java isolates were observed with low-level resistance to ciprofloxacin but without high level resistance to nalidixic acid. This phenotype has been described to be typical for plasmid mediated quinolone resistance genes (*qnr*) in *Salmonella enterica.* Moreover one isolate was observed in January 2008 that was both cefotaxime resistant and also showed the *qnr*-phenotype. This indicates the presence of plasmid mediated ESBLs and quinolone resistance genes in one isolate.
Molecular identification identified the presence of both $bla_{\text{CTX-M-9}}$ and *qnr*A1 on a large plasmid in one *S.* Java isolate and *qnr*B5 in two other *S.* Java isolates.

This indicates that ESBLs are well established in the GI-tract of broiler chickens and that transmission has occurred between *E. coli* and *Salmonella.* The detection of mobile quinolone resistance genes, partly linked to and ESBL is a worrisome development given the high selection pressure in poultry production.

Figure 29. Genes and plasmids detected in *S.* Java isolates



Appendix III. Materials and Methods

Salmonella enterica

A total of 16.861 isolates were tested for antimicrobial susceptibility between 1999-2007 (table 31). Human isolates (N=9155) concerned a selection from first isolates sent to the Dutch National Institute of Public Health (RIVM) by the regional public health laboratories. All strains were the first isolates recovered from patients with salmonellosis. The majority of the isolates from pigs (N=1124) and cattle, including calves (N=600) were sent to the RIVM by the Animal Health Service from a diversity of surveillance programs and clinical Salmonella infections. Those from chickens (broilers, including poultry products, N=1149; layers, reproduction animals and eggs, N=827) concerned mainly nonclinical Salmonella infections derived from a diversity of monitoring programs on the farm, slaughterhouses and at retail.. The majority of isolates from layers in 2005 concerned those from the Dutch component of the EU-baseline study. Isolates from a diversity of other sources have been analysed as well (animal fodder and human food products; other animals from animal husbandry and pets, samples from the environment, etc.).

Table 31. Number of Salmonella isolates tested for susceptibility from 1999 – 2007 in the Netherlands.

	Total	1999	2000	2001	2002	2003	2004	2005	2006	2007
Human	9155	674	349	1055	862	1338	1339	1176	1273	1089
Pig	1124	31	195	114	168	127	119	120	115	135
Cattle	600	18	28	56	33	23	106	90	159	87
Chicken (misc.)	850	0	10	174	172	160	29	30	116	159
Broilers (faeces/meat)	1149	68	110	143	212	206	110	82	164	54
Layers/Repro/Eggs	827	93	86	62	56	88	91	232	75	44
Other sources	3156	0	9	309	330	446	473	603	535	451
Total	16861	884	787	1913	1833	2388	2267	2333	2437	2019

Representativeness of percentages of resistance for humans or animals over all types

In principal, if isolates are selected randomly from a source the percentage of resistant strains within a source can be computed straightforwardly. Standard statistical considerations would apply to indicate significant differences between years and between animal and human sources. Table 32 shows that quite substantial numbers are needed to indicate significant differences in resistance percentages less than 10%. However, resistance strongly depends on Salmonella type and many different types are involved; a cocktail of types that differs between sources and that may differ between years. Moreover, low numbers tested and incidentally missed, or selected types with rare antibiograms, may influence the resulting resistance percentages. Finally the source definition in itself may be biased, as the reason for sending-in isolates, especially from cattle and pigs, is often unknown. This explains many of the irregularities between years.

Table 32. Power analysis to show the sample sizes needed to indicate significant differences in resistance percentages between groups (for example between years or between human and animal sources).

Level of significance = 0,05 and Power = 0,7			
R-group 1	R-group 2	Difference	N1=N2
40%	30%	10%	287
30%	20%	10%	251
20%	10%	10%	211
70%	50%	20%	111
60%	40%	20%	95
50%	30%	20%	84
40%	20%	20%	70
30%	10%	20%	59
60%	30%	30%	23

E. coli, *E. faecium*, *E. faecalis* and *Campylobacter* spp. isolated from slaughter pigs and broilers
E. coli and *E. faecium*, *E. faecalis* and *Campylobacter* spp. were isolated from faecal samples taken from healthy animals at slaughter by the Food and Consumer Product Safety Authority as part of the national control programs. Samples were taken at slaughterhouses or at farms. For isolation of the above mentioned organisms one faecal sample was taken for each epidemiological unit (farm, flock or group of animals) aseptically, or the caeca collected (broilers). At the laboratory the samples were directly 1:10 diluted in buffered peptone solution with 20% glycerol and stored at –20°C. *E. coli*, *E. faecium*, *E. faecalis* and *Campylobacter* spp. were isolated directly after arrival of the samples at CVI-Lelystad or the Food and Consumer Product Safety Authority in Zutphen. For *E. coli* MacConkey agar and for the enterococci Slanetz and Bartley agar was inoculated with cotton swabs (*E. coli*), or a50 µl of a serial dilution (enterococci). A colony with typical morphology was subcultured to obtain a pure culture and stored at –80°C in buffered peptone water with 20% glycerol. *E. coli* was identified biochemically. The final identification of the enterococci was done with Polymerase Chain Reaction (PCR) as described by Dutka Malen in 1995.
For isolation of Campylobacter CCDA-agar with 32 µg/ml cefoperazone and 10 µg/ml amphotericin B to inhibit growth of Gram-negative bacteria and fungi, was directly inoculated with a cotton swab. All campylobacters were typed with PCR to the species level. Only *C. jejuni* and *C. coli* were tested for their susceptibility. All other spp. were excluded from the programme.

E. coli, *E. faecium* and *E. faecalis* isolated from raw meat products of food-animals

For isolation of all bacterial species raw meat products were rinsed with Buffered Peptone Water (BPW). For *E. coli* 10 ml BPW rinse was enriched in 90 MacConkey-, or Laurylsulphate broth. After overnight aerobic incubation at 44°C the broth was subcultured on Coli-ID agar (24 h at 44°C). For enterococci 10 ml BPW rinse was enriched in 90 ml Azide Dextrose broth. After overnight aerobic incubation at 44°C, the broth was subcultured on Slanetz and Bartley agar for 48 hrs at 44°C. Identification was done biochemically.

Shigella toxin producing *E. coli* O157 (STEC)

For STEC both human and animal strains were combined. All sorbitol negative human strains from all medical microbiological laboratories in the Netherlands were sent to RIVM for serovar O157 confirmation and further typing. The animal strains were partly isolated in the monitoring programme of farm-animals of VWA/RIVM. These samples were taken at farms from faeces of healthy animals. One isolate per farm was included. Isolates from non-human sources included strains isolated from samples taken in an attempt to trace a human infection.

Bovine mastitis pathogens *E. coli*, coliform bacteria, *S. aureus*, coagulase-negative staphylococci, *S. uberis* and *S. dysgalactiae*.

Annually at the Animal Health Service large numbers of milk samples from clinical cases of bovine mastitis are sent in for bacteriological examination. From the isolates a selection of approximately 100 strains of *E. coli*, coliform bacteria, *S. aureus*, coagulase-negative staphylococci, *S. uberis* and *S. dysgalactiae* were sent to CVI-Lelystad for MIC-determinations. Inclusion criteria for the strains were: a maximum of one isolate per species per farm, only pure cultures were included after direct inoculations from the milk samples on agar plates, except for *S. aureus* for which species also pure cultures after broth enrichment were included.

Susceptibility tests

Susceptibility was tested quantitatively with the broth micro dilution test with cation-adjusted Mueller Hinton broth according to ISO standard 20776-1-2006 or CLSI guidelines M31-A3 for Campylobacter spp.. For broth microdilution, microtitre trays were used with dehydrated dilution ranges of custom made panels of antibiotics. Trek Diagnostic Systems, in the UK, manufactured these microtitre trays. ATCC strains *E. coli* 25922 and *E. faecalis* 29212 were used daily to monitor the quality of the results. For quality control of the results of campylobacters, *C. jejuni* ATCC 33560 was used as control strain. The MICs were defined as the lowest concentration without visible growth. Strains with MIC's higher than the MIC-breakpoints were considered resistant. Percentages of resistance were calculated. For Salmonella, the indicator organisms *E. coli* and enterococci and Campylobacter spp. EUCAST epidemiological cut-off values were used as prescribed by EFSA[19][20] (table 34). For the animal pathogens clinical breakpoints were used (CLSI M31-A3, M100-S17) as listed in table 34.

Data interpretation needs to take into account that for some antibiotics the cut-off values are substantially lower than the previously used clinical breakpoints, which may have affected the level of the resistance percentages. These percentages indicate the acquisition of resistance in intrinsically susceptible bacteria population as an effect of determinants like antibiotic usage. They cannot directly be translated in therapeutic failure, when antibiotics would be used to treat infection with those organisms.

[19]Report from the Task Force of Zoonoses Data Collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers, *the EFSA Journal* (2007), 96,1-46.

[20] Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal *Escherichia coli* and *Enterococcus* spp. from food animals 1. *The EFSA Journal* (2008) 141: 1-44

Table 33. Epidemiological cut-off values (mg/L) used for the classification of *Salmonella*, *E. coli* (indicator organism), *Campylobacter* spp. and enterococci. Isolates with MIC-values higher than those presented in this table are considered resistant.

	Salmonella	*E. coli*	*C. jejuni*	*C. coli*	*E. faecium*	*E. faecalis*
Amoxicillin	4	8	16	8	-	-
Cefotaxime	0.5	0.25	-	-	-	-
Ceftazidime	2	0.5	-	-	-	-
Streptomycin	-	16	2	4	2000	2000
Gentamicin	2	2	1	2	500	500
Kanmycin	8	8	-	-	-	-
Neomycin	4	8	1	2	-	-
Tetracycline	8	8	2	2	2	2
Sulphamethoxazole	256*	256*	256	32	-	-
Trimethoprim	2	2	-	-	-	-
Trim/sulphamethoxazole	2/38	2/38	16/304	4/76	-	-
Nalidixic acid	16	16	16	32	-	-
Ciprofloxacin	0.06	0.06	1	1	4	4
Chloramphenicol	16	16	16	16	32	32
Florfenicol	16	16	-	-	-	-
Vancomycin	-	-	-	-	4	4
Flavomycin	-	-	-	-	-	16
Quinu/dalfopristin	-	-	-	-	1	32
Erythromycin	-	-	4	16	4	4
Clarithromycin	-	-	8	32	-	-
Tulathromycin	-	-	16	16	-	-
Linezolid	-	-	-	-	4*	4*
Colistin	8	8	-	-	-	-
Salinomycin	-	-	-	-	4	4

* CLSI breakpoint

Table 34. MIC-breakpoints (mg/L) used for the classification of *E. coli* and coliform bacteria (mastitis), *P. multocida*, *M. haemolytica*, *S. aureus*, coagulase negative staphylococci (CNS) and streptococci. Isolates with MIC-values higher than those presented in this table are considered resistant.

	E. coli/coliform bactteria	*P. multocida*	*M. haemolytica*	*S. aureus*	*CNS*	*Streptococcus* spp.
Penicillin	-	-	-	0.125	0.125	2
Oxacillin	-			2	*	-
Ampicillin	16	16	16	-	-	-
Amox-clavulanic acid	16	-	-	4	4	4
Cephalothin	-	-	-	16	16	16
Cefuroxime	16	-	-	-	-	-
Cefquinome	4	-	-	-	-	-
Ceftiofur	-	4	4	-	-	-
Cefoperazone	32	-	-	-	-	-
Streptomycin	32	-	-	16	16	-
Spectonomycin	-	64	64	-	-	-
Gentamicin	8	4	4	-	-	-
Neomycin	16	16	16	16	16	-
Kanamycin	32	-	-	16	16	
Tetracycline	8	8	8	8	8	8
Trim/sulphamethoxazole	2/38	2/38	2/38	2/38	2/38	2/38
Flumequine	-	4	4	-	-	-
Enrofloxacin	2	1	1	-	-	-
Chloramphenicol	-	16	16	-	-	-
Florfenicol	-	4	4	-	-	-
Erythromycin	-	-	-	4	4	0.5
Pirlimycin	-	-	-	2	2	2
Clindamycin	-	-	-	2	2	-

* Only mecA positive isolates were classified resistant, this equals MIC ≥ 4 mg/L

Appendix IV. Annexes to Chapter 1 Usage of antibiotics in animal husbandry in the Netherlands

Annex 1a Average number of daily dosages dairy cows per animal year

	ANIMAL SPECIES		DAIRY CATTLE					
			2006			2007		
	NUMBER OF FARMS		35			35		
	TOTAL daily dosages per animal year		5.5			5.4		
			mam.	oral	other	mam.	oral	other
group		**sum**	**3.48**	**0.08**	**1.86**	**2.86**	**0.42**	**2.13**
Cephalosporines	Cefquinome		0.18	0	0.03	0.27	0	0.02
	Ceftiofur		0	0	0.45	0	0	0.55
	Cefapirin		0	0	0.02	0	0	0.03
	Cefoperazone		0.03	0	0	0.07	0	0
	Cefalexin		0.14	0	0	0.05	0	0
			0.35	0	0.5	0.39	0	0.6
Penicillin	Benzylpenicillin		0	0	0.36	0	0	0.3
	Ampicillin		0	0	0.03	0	0	0.09
	Amoxicillin		0	0	0	0	0	0
	Cloxacillin		1.15	0	0	0.34	0	0
	Fenoxymethylpenicillin		0	0	0	0	0	0
			1.15	0	0.39	0.34	0	0.39
Macrolides	Erythromycin		0	0	0	0	0	0
	Tylosin		0	0	0.13	0	0	0.16
	Tilmicosin		0	0	0	0	0	0
	Tulathromycin		0	0	0.01	0	0	0.03
			0	0	0.14	0	0	0.19
Quinolones	Danofloxacin		0	0	0	0	0	0
	Enrofloxacin		0	0	0.04	0	0	0.06
	Flumequine		0	0	0	0	0	0
	Difloxacin		0	0	0	0	0	0
	Marbofloxacin		0	0	0	0	0	0
			0	0	0.04	0	0	0.06
Sulfanomides and trimethoprim	Trimethoprim-sulfachloorpyridazin		0	0	0	0	0	0
	Trimethoprim-sulfadiazin		0	0.02	0.06	0	0.01	0.06
	Trimethoprim-sulfadoxin		0	0	0.1	0	0	0.11
	Trimethoprim-sulfamethoxazole		0	0	0	0	0	0
	Sulfadimidine		0	0	0	0	0	0
	Sulfaclozine Na		0	0	0	0	0	0
	Sulfaquinoxalin		0	0	0	0	0	0
			0	0.02	0.16	0	0.01	0.17
Tetracyclines	Tetracycline		0	0	0.02	0	0	0.01
	Chloortetracycline		0	0	0	0	0	0
	Doxycycline		0	0.03	0	0	0.33	0
	Oxytetracycline		0	0	0.4	0	0.01	0.45
			0	0.03	0.42	0	0.34	0.46
Aminoglycocides	Gentamicin		0	0	0.01	0	0	0.01
	Neomycin		0	0	0	0	0	0
			0	0	0.01	0	0	0.01
Combinations	Amoxicillin-clavulanic acid		0.76	0	0	1.11	0	0
	Amoxicillin-colistin		0	0	0	0	0	0
	Ampicillin-colistin		0	0	0	0	0	0
	Ampicillin-cloxacillin		0.25	0	0	0.2	0	0
	Dihydrostreptomycin-benzylpenicillin		0	0	0.06	0	0	0.07
	Dihydrostreptomycin-benzylpenicillin-nafcilline		0.53	0	0	0.44	0	0.01
	Lincomycin-neomycin		0.14	0	0	0.16	0	0
	Lincomycin-spectinomycin		0	0	0	0	0.02	0
	Neomycin-benzylpenicillin		0.3	0	0.13	0.22	0	0.14
	Dihydrostreptomycin		0	0	0	0	0	0
			1.98	0	0.19	2.13	0.02	0.22
Others	Florfenicol		0	0	0.01	0	0	0.03
	Lincomycin		0	0	0	0	0.02	0
	Colistin		0	0.03	0	0	0.03	0
	Tiamulin		0	0	0	0	0	0
	Pirlimycin		0	0	0	0	0	0
			0	0.03	0.01	0	0.05	0.03

Annex 1b Average number of daily dosages fattening pigs per animal year

	ANIMAL SPECIES	FATTENING PIGS					
		2006			2007		
	NUMBER OF FARMS	31			31		
	TOTAL daily dosages per animal year	15.0			17.0		
		mam.	oral	other	mam.	oral	other
group	**sum**	**0**	**14.33**	**0.65**	**0**	**15.92**	**1.01**
Cephalosporines	Cefquinome	0	0	0	0	0	0
	Ceftiofur	0	0	0.02	0	0	0
	Cefapirin	0	0	0	0	0	0
	Cefoperazone	0	0	0	0	0	0
	Cefalexin	0	0	0	0	0	0
		0	0	0.02	0	0	0
Penicillin	Benzylpenicillin	0	0	0.21	0	0	0.27
	Ampicillin	0	0	0.08	0	0	0.11
	Amoxicillin	0	0.03	0	0	0.02	0
	Cloxacillin	0	0	0	0	0	0
	Fenoxymethylpenicillin	0	0	0	0	0	0
		0	0.03	0.29	0	0.02	0.38
Macrolides	Erythromycin	0	0	0	0	0	0
	Tylosin	0	1.23	0.04	0	2.02	0.09
	Tilmicosin	0	0	0	0	0.13	0
	Tulathromycin	0	0	0	0	0	0.02
		0	1.23	0.04	0	2.15	0.11
Quinolones	Danofloxacin	0	0	0	0	0	0
	Enrofloxacin	0	0	0.01	0	0	0.01
	Flumequine	0	0	0	0	0	0
	Difloxacin	0	0	0	0	0	0
	Marbofloxacin	0	0	0	0	0	0
		0	0	0.01	0	0	0.01
Sulfanomides and trimethoprim	Trimethoprim-sulfachloorpyridazin	0	0	0	0	0	0
	Trimethoprim-sulfadiazin	0	1.2	0	0	0.78	0
	Trimethoprim-sulfadoxin	0	0	0	0	0	0
	Trimethoprim-sulfamethoxazole	0	0.43	0	0	0.28	0
	Sulfadimidine	0	0	0	0	0	0
	Sulfaclozine Na	0	0	0	0	0	0
	Sulfaquinoxalin	0	0	0	0	0	0
		0	1.63	0	0	1.06	0
Tetracyclines	Tetracycline	0	0	0	0	0	0
	Chloortetracycline	0	0	0	0	0	0
	Doxycycline	0	2	0	0	3.37	0
	Oxytetracycline	0	8.97	0.24	0	6.12	0.39
		0	10.97	0.24	0	9.49	0.39
Aminoglycocides	Gentamicin	0	0	0	0	0	0
	Neomycin	0	0	0	0	0	0
		0	0	0	0	0	0
Combinations	Amoxicillin-clavulanic acid	0	0	0	0	0	0
	Amoxicillin-colistin	0	0.29	0.02	0	0.06	0.03
	Ampicillin-colistin	0	0	0	0	0	0
	Ampicillin-cloxacillin	0	0	0	0	0	0
	Dihydrostreptomycin-benzylpenicillin	0	0	0.02	0	0	0.06
	Dihydrostreptomycin-benzylpenicillin-nafcilline	0	0	0	0	0	0
	Lincomycin-neomycin	0	0	0	0	0	0
	Lincomycin-spectinomycin	0	0.12	0	0	0.17	0
	Neomycin-benzylpenicillin	0	0	0	0	0	0.01
	Dihydrostreptomycin	0	0	0	0	0	0
		0	0.41	0.04	0	0.23	0.1
Others	Florfenicol	0	0	0.01	0	0	0.01
	Lincomycin	0	0	0	0	0	0
	Colistin	0	0.05	0	0	2.81	0
	Tiamulin	0	0.01	0	0	0.16	0.01
	Pirlimycin	0	0	0	0	0	0
		0	0.06	0.01	0	2.97	0.02

Annex 1c Average number of daily dosages in sows/piglets per animal year

	ANIMAL SPECIES	SOWS /PIGLETS					
		2006			2007		
	NUMBER OF FARMS	30			30		
	TOTAL daily dosages per animal year	26.5			22.4		
		mam.	oral	other	mam.	oral	other
group	**sum**	**0**	**22.53**	**3.93**	**0**	**18.41**	**3.99**
Cephalosporines	Cefquinome	0	0	0.02	0	0	0.04
	Ceftiofur	0	0	0.11	0	0	0.11
	Cefapirin	0	0	0	0	0	0
	Cefoperazone	0	0	0	0	0	0
	Cefalexin	0	0	0	0	0	0
		0	0	0.13	0	0	0.15
Penicillin	Benzylpenicillin	0	0	0.83	0	0	0.82
	Ampicillin	0	0.29	0.39	0	0.21	0.62
	Amoxicillin	0	4.32	0.07	0	3.6	0.04
	Cloxacillin	0	0	0	0	0	0
	Fenoxymethylpenicillin	0	0	0	0	0	0
		0	4.61	1.29	0	3.81	1.48
Macrolides	Erythromycin	0	0	0	0	0	0
	Tylosin	0	0.69	0.01	0	0.23	0.01
	Tilmicosin	0	0.75	0	0	0.55	0
	Tulathromycin	0	0	1.15	0	0	0.84
		0	1.44	1.16	0	0.78	0.85
Quinolones	Danofloxacin	0	0	0	0	0	0
	Enrofloxacin	0	0	0.03	0	0	0.02
	Flumequine	0	0	0	0	0	0
	Difloxacin	0	0	0	0	0	0
	Marbofloxacin	0	0	0.01	0	0	0.02
		0	0	0.04	0	0	0.04
Sulfanomides and trimethoprim	Trimethoprim-sulfachloorpyridazin	0	0	0	0	0	0
	Trimethoprim-sulfadiazin	0	4.78	0.22	0	4.88	0.23
	Trimethoprim-sulfadoxin	0	0	0.12	0	0	0.12
	Trimethoprim-sulfamethoxazole	0	1.49	0.01	0	0.8	0
	Sulfadimidine	0	0	0	0	0	0
	Sulfaclozine Na	0	0	0	0	0	0
	Sulfaquinoxalin	0	0	0	0	0	0
		0	6.27	0.35	0	5.68	0.35
Tetracyclines	Tetracycline	0	0	0	0	0	0
	Chloortetracycline	0	0	0	0	0	0
	Doxycycline	0	3.29	0	0	1.05	0
	Oxytetracycline	0	5.35	0.25	0	4.93	0.22
		0	8.64	0.25	0	5.98	0.22
Aminoglycocides	Gentamicin	0	0.06	0.01	0	0.02	0
	Neomycin	0	0	0	0	0	0
		0	0.06	0.01	0	0.02	0
Combinations	Amoxicillin-clavulanic acid	0	0	0	0	0	0
	Amoxicillin-colistin	0	0.02	0.13	0	0.02	0.13
	Ampicillin-colistin	0	0	0	0	0	0
	Ampicillin-cloxacillin	0	0	0	0	0	0
	Dihydrostreptomycin-benzylpenicillin	0	0	0.55	0	0	0.62
	Dihydrostreptomycin-benzylpenicillin-nafcilline	0	0	0	0	0	0
	Lincomycin-neomycin	0	0	0	0	0	0
	Lincomycin-spectinomycin	0	0.09	0	0	0.17	0.02
	Neomycin-benzylpenicillin	0	0	0.01	0	0	0.11
	Dihydrostreptomycin	0	0	0	0	0	0
		0	0.11	0.69	0	0.19	0.88
Others	Florfenicol	0	0	0.01	0	0	0.02
	Lincomycin	0	0	0	0	0.19	0
	Colistin	0	1.26	0	0	1.64	0
	Tiamulin	0	0.14	0	0	0.12	0
	Pirlimycin	0	0	0	0	0	0
		0	1.4	0.01	0	1.95	0.02

Annex 1d Average number of daily dosages in broilers per animal year

	ANIMAL SPECIES	BROILERS					
		2006			2007		
	NUMBER OF FARMS	27			27		
	TOTAL daily dosages per animal year	25.4			30.2		
		mam.	oral	other	mam.	oral	other
group		**sum**	**0**	**25.42**	**0**	**0**	**30.2**	**0**
Cephalosporines	Cefquinome	0	0	0	0	0	0
	Ceftiofur	0	0	0	0	0	0
	Cefapirin	0	0	0	0	0	0
	Cefoperazone	0	0	0	0	0	0
	Cefalexin	0	0	0	0	0	0
		0	0	0	0	0	0
Penicillin	Benzylpenicillin	0	0	0	0	0	0
	Ampicillin	0	0.25	0	0	0.38	0
	Amoxicillin	0	3.42	0	0	6.74	0
	Cloxacillin	0	0	0	0	0	0
	Fenoxymethylpenicillin	0	0.94	0	0	1.36	0
		0	4.61	0	0	8.48	0
Macrolides	Erythromycin	0	0	0	0	0	0
	Tylosin	0	1.01	0	0	1.52	0
	Tilmicosin	0	0	0	0	0	0
	Tulathromycin	0	0	0	0	0	0
		0	1.01	0	0	1.52	0
Quinolones	Danofloxacin	0	0	0	0	0	0
	Enrofloxacin	0	0.17	0	0	0.48	0
	Flumequine	0	8.81	0	0	6.71	0
	Difloxacin	0	0	0	0	0	0
	Marbofloxacin	0	0	0	0	0	0
		0	8.98	0	0	7.19	0
Sulfanomides and trimethoprim	Trimethoprim-sulfachloorpyridazin	0	1.95	0	0	1.55	0
	Trimethoprim-sulfadiazin	0	0	0	0	0	0
	Trimethoprim-sulfadoxin	0	0	0	0	0	0
	Trimethoprim-sulfamethoxazole	0	1.37	0	0	1.32	0
	Sulfadimidine	0	0.41	0	0	0.39	0
	Sulfaclozine Na	0	0	0	0	0	0
	Sulfaquinoxalin	0	0.15	0	0	0	0
		0	3.88	0	0	3.26	0
Tetracyclines	Tetracycline	0	0	0	0	0	0
	Chloortetracycline	0	0	0	0	0	0
	Doxycycline	0	1.91	0	0	3.88	0
	Oxytetracycline	0	1.52	0	0	0.91	0
		0	3.43	0	0	4.79	0
Aminoglycocides	Gentamicin	0	0	0	0	0	0
	Neomycin	0	3.19	0	0	4.67	0
		0	3.19	0	0	4.67	0
Combinations	Amoxicillin-clavulanic acid	0	0	0	0	0	0
	Amoxicillin-colistin	0	0	0	0	0	0
	Ampicillin-colistin	0	0	0	0	0	0
	Ampicillin-cloxacillin	0	0	0	0	0	0
	Dihydrostreptomycin-benzylpenicillin	0	0	0	0	0	0
	Dihydrostreptomycin-benzylpenicillin-nafcilline	0	0	0	0	0	0
	Lincomycin-neomycin	0	0	0	0	0	0
	Lincomycin-spectinomycin	0	0.32	0	0	0.14	0
	Neomycin-benzylpenicillin	0	0	0	0	0	0
	Dihydrostreptomycin	0	0	0	0	0	0
		0	0.32	0	0	0.14	0
Others	Florfenicol	0	0	0	0	0	0
	Lincomycin	0	0	0	0	0	0
	Colistin	0	0	0	0	0.15	0
	Tiamulin	0	0	0	0	0	0
	Pirlimycin	0	0	0	0	0	0
		0	0	0	0	0.15	0

Annex 2a Average number of grams active ingredient in dairy cows per animal year

	ANIMAL SPECIES	DAIRY CATTLE					
		2006			2007		
	NUMBER OF FARMS	35			35		
	TOTAL daily dosages per animal year	57.8			54.9		
		mam.	oral	other	mam.	oral	other
group	**sum**	**1.62**	**48.06**	**8.14**	**1.66**	**43.38**	**9.88**
Cephalosporines	Cefquinome	0.01	0	0.03	0.02	0	0.02
	Ceftiofur	0	0	0.27	0	0	0.33
	Cefapirin	0	0	0.01	0	0	0.02
	Cefoperazone	0.01	0	0	0.02	0	0
	Cefalexin	0.03	0	0	0.01	0	0
		0.05	0	0.31	0.05	0	0.37
Penicillin	Benzylpenicillin	0	0	2.1	0	0	1.71
	Ampicillin	0	0	0.25	0	0	1.04
	Amoxicillin	0	2.93	0.05	0	4.16	0.02
	Cloxacillin	0.64	0	0	0.76	0	0
	Fenoxymethylpenicillin	0	0	0	0	0	0
		0.64	2.93	2.4	0.76	4.16	2.77
Macrolides	Erythromycin	0	0	0.01	0	0	0
	Tylosin	0	3.52	0.62	0	3.9	0.69
	Tilmicosin	0	0	0.01	0	2.16	0.01
	Tulathromycin	0	0	0.01	0	0	0.01
		0	3.52	0.65	0	6.06	0.71
Quinolones	Danofloxacin	0	0	0	0	0	0
	Enrofloxacin	0	0	0.09	0	-0.04	0.14
	Flumequine	0	0.54	0	0	2.79	0
	Difloxacin	0	0	0	0	0	0
	Marbofloxacin	0	0	0	0	0	0
		0	0.54	0.09	0	2.75	0.14
Sulfanomides and trimethoprim	Trimethoprim-sulfachloorpyridazin	0	0	0	0	0	0
	Trimethoprim-sulfadiazin	0	7.55	0.78	0	2.55	0.8
	Trimethoprim-sulfadoxin	0	0	1.06	0	0	1.06
	Trimethoprim-sulfamethoxazole	0	8.73	0	0	5.57	0
	Sulfadimidine	0	0	0	0	0	0
	Sulfaclozine Na	0	0	0	0	0	0
	Sulfaquinoxalin	0	2.42	0	0	0	0
		0	18.7	1.84	0	8.12	1.86
Tetracyclines	Tetracycline	0	0	0.03	0	0	0.02
	Chloortetracycline	0	0	0	0	0	0
	Doxycycline	0	3.89	0	0	14.31	0
	Oxytetracycline	0	17.33	1.06	0	7.63	2.2
		0	21.22	1.09	0	21.94	2.22
Aminoglycocides	Gentamicin	0	0	0.02	0	0	0.02
	Neomycin	0	0.72	0	0	0	0
		0	0.72	0.02	0	0	0.02
Combinations	Amoxicillin-clavulanic acid	0.19	0.01	0	0.28	0	0
	Amoxicillin-colistin	0	0	0.02	0	0	0.07
	Ampicillin-colistin	0.07	0	0	0.05	0	0
	Ampicillin-cloxacillin	0	0	0	0	0	0
	Dihydrostreptomycin-benzylpenicillin	0	0	0.52	0	0	0.58
	Dihydrostreptomycin-benzylpenicillin-nafcilline	0.2	0	0.04	0.16	0	0.04
	Lincomycin-neomycin	0.06	0	0	0.07	0	0
	Lincomycin-spectinomycin	0	0.11	0.02	0	0.12	0.01
	Neomycin-benzylpenicillin	0.41	0	0.96	0.29	0	0.9
	Dihydrostreptomycin	0	0	0	0	0	0
		0.93	0.12	1.56	0.85	0.12	1.6
Others	Florfenicol	0	0	0.18	0	0	0.19
	Lincomycin	0	0	0	0	0.04	0
	Colistin	0	0.31	0	0	0.19	0
	Tiamulin	0	0	0	0	0	0
	Pirlimycin	0	0	0	0	0	0
		0	0.31	0.18	0	0.23	0.19

Annex 2b Average number of grams active ingredient in fattening pigs per animal year

	ANIMAL SPECIES	FATTENING PIGS					
		2006			2007		
	NUMBER OF FARMS	31			31		
	TOTAL daily dosages per animal year	42.1			59.2		
		mam.	oral	other	mam.	oral	other
group	**sum**	**0.00**	**41.30**	**0.79**	**0.00**	**57.98**	**1.17**
Cephalosporines	Cefquinome	0	0	0	0	0	0
	Ceftiofur	0	0	0.01	0	0	0
	Cefapirin	0	0	0	0	0	0
	Cefoperazone	0	0	0	0	0	0
	Cefalexin	0	0	0	0	0	0
		0	0	0.01	0	0	0
Penicillin	Benzylpenicillin	0	0	0.14	0	0	0.18
	Ampicillin	0	1.47	0.15	0	0.06	0.16
	Amoxicillin	0	1.26	0.03	0	2.28	0.02
	Cloxacillin	0	0	0	0	0	0.02
	Fenoxymethylpenicillin	0	2.5	0	0	7.57	0
		0	5.23	0.32	0	9.91	0.38
Macrolides	Erythromycin	0	0	0	0	0	0
	Tylosin	0	5.24	0.02	0	10.25	0.07
	Tilmicosin	0	0.19	0	0	0.13	0
	Tulathromycin	0	0	0	0	0	0
		0	5.43	0.02	0	10.38	0.07
Quinolones	Danofloxacin	0	0	0	0	0	0
	Enrofloxacin	0	0.35	0	0	0.53	0.03
	Flumequine	0	0.09	0	0	0.39	0
	Difloxacin	0	0	0	0	0	0
	Marbofloxacin	0	0	0	0	0	0
		0	0.44	0	0	0.92	0.03
Sulfanomides and trimethoprim	Trimethoprim-sulfachloorpyridazin	0	0	0	0	0	0
	Trimethoprim-sulfadiazin	0	2.76	0.07	0	2.08	0.06
	Trimethoprim-sulfadoxin	0	0	0.01	0	0	0.01
	Trimethoprim-sulfamethoxazole	0	1.11	0	0	0.73	0
	Sulfadimidine	0	0	0	0	0	0
	Sulfaclozine Na	0	0.21	0	0	0	0
	Sulfaquinoxalin	0	0	0	0	4.14	0
		0	4.08	0.08	0	6.95	0.07
Tetracyclines	Tetracycline	0	0	0	0	0	0
	Chloortetracycline	0	0	0	0	0	0
	Doxycycline	0	5.7	0	0	12.3	0
	Oxytetracycline	0	19.49	0.15	0	15.87	0.23
		0	25.19	0.15	0	28.17	0.23
Aminoglycocides	Gentamicin	0	0	0	0	0	0.09
	Neomycin	0	0.21	0	0	0.35	0
		0	0.21	0	0	0.35	0.09
Combinations	Amoxicillin-clavulanic acid	0	0	0.01	0	0	0
	Amoxicillin-colistin	0	0.47	0.03	0	0.1	0.03
	Ampicillin-colistin	0	0	0	0	0	0
	Ampicillin-cloxacillin	0	0	0	0	0	0
	Dihydrostreptomycin-benzylpenicillin	0	0	0.15	0	0	0.21
	Dihydrostreptomycin-benzylpenicillin-nafcilline	0	0	0	0	0	0.01
	Lincomycin-neomycin	0	0	0	0	0	0
	Lincomycin-spectinomycin	0	0.02	0	0	0.06	0
	Neomycin-benzylpenicillin	0	0	0.01	0	0	0.01
	Dihydrostreptomycin	0	0	0	0	0	0
		0	0.49	0.2	0	0.16	0.26
Others	Florfenicol	0	0	0.01	0	0	0.03
	Lincomycin	0	0	0	0	0	0
	Colistin	0	0.23	0	0	1.08	0
	Tiamulin	0	0	0	0	0.06	0.01
	Pirlimycin	0	0	0	0	0	0
		0	0.23	0.01	0	1.14	0.04

Annex 2c Average number of grams active ingredient in sows/piglets per animal year

	ANIMAL SPECIES	SOWS /PIGLETS					
		2006			2007		
	NUMBER OF FARMS	30			30		
	TOTAL daily dosages per animal year	140.7			142.8		
		mam.	oral	other	mam.	oral	other
group	**sum**	**0.00**	**129.47**	**10.57**	**0.00**	**131.31**	**11.10**
Cephalosporines	Cefquinome	0	0	0.01	0	0	0.02
	Ceftiofur	0	0	0.16	0	0	0.15
	Cefapirin	0	0	0	0	0	0
	Cefoperazone	0	0	0	0	0	0
	Cefalexin	0	0	0	0	0	0
		0	0	0.17	0	0	0.17
Penicillin	Benzylpenicillin	0	0	1.74	0	0	1.9
	Ampicillin	0	2.04	1.96	0	1.54	2.03
	Amoxicillin	0	17.71	0.53	0	14.95	0.4
	Cloxacillin	0	0	0	0	0	0
	Fenoxymethylpenicillin	0	0	0	0	0	0
		0	19.75	4.23	0	16.49	4.33
Macrolides	Erythromycin	0	0	0	0	0	0
	Tylosin	0	5.39	0.02	0	5.16	0.03
	Tilmicosin	0	2.81	0	0	2.24	0
	Tulathromycin	0	0	0.17	0	0	0.13
		0	8.2	0.19	0	7.4	0.16
Quinolones	Danofloxacin	0	0	0	0	0	0
	Enrofloxacin	0	0	0.03	0	0	0.02
	Flumequine	0	0	0	0	0	0
	Difloxacin	0	0	0	0	0	0
	Marbofloxacin	0	0	0	0	0	0.02
		0	0	0.03	0	0	0.04
Sulfanomides and trimethoprim	Trimethoprim-sulfachloorpyridazin	0	0	0	0	0	0
	Trimethoprim-sulfadiazin	0	33.32	1.36	0	37.71	1.15
	Trimethoprim-sulfadoxin	0	0	0.65	0	0	0.68
	Trimethoprim-sulfamethoxazole	0	10.47	0.1	0	6.51	0.04
	Sulfadimidine	0	0	0	0	0	0
	Sulfaclozine Na	0	0	0	0	0	0
	Sulfaquinoxalin	0	0	0	0	0	0
		0	43.79	2.11	0	44.22	1.87
Tetracyclines	Tetracycline	0	0	0	0	0	0
	Chloortetracycline	0	0	0	0	0	0
	Doxycycline	0	13.66	0	0	8.68	0
	Oxytetracycline	0	41.72	0.73	0	50.97	0.66
		0	55.38	0.73	0	59.65	0.66
Aminoglycocides	Gentamicin	0	0.02	0.01	0	0.01	0
	Neomycin	0	0	0	0	0	0
		0	0.02	0.01	0	0.01	0
Combinations	Amoxicillin-clavulanic acid	0	0	0	0	0	0
	Amoxicillin-colistin	0	0.11	0.44	0	0.15	0.44
	Ampicillin-colistin	0	0	0	0	0	0
	Ampicillin-cloxacillin	0	0	0	0	0	0
	Dihydrostreptomycin-benzylpenicillin	0	0	2.53	0	0	2.87
	Dihydrostreptomycin-benzylpenicillin-nafcilline	0	0	0	0	0	0
	Lincomycin-neomycin	0	0	0	0	0	0
	Lincomycin-spectinomycin	0	0.15	0.02	0	0.24	0.08
	Neomycin-benzylpenicillin	0	0	0.04	0	0	0.37
	Dihydrostreptomycin	0	0	0	0	0	0
		0	0.26	3.03	0	0.39	3.76
Others	Florfenicol	0	0	0.07	0	0	0.11
	Lincomycin	0	0.01	0	0	0.43	0
	Colistin	0	1.98	0	0	2.63	0
	Tiamulin	0	0.08	0	0	0.09	0
	Pirlimycin	0	0	0	0	0	0
		0	2.07	0.07	0	3.15	0.11

Annex 2d Average number of grams active ingredient broilers per animal year

	ANIMAL SPECIES	BROILERS					
		2006			2007		
	NUMBER OF FARMS	27			27		
	TOTAL daily dosages per animal year	0.6			0.7		
		mam.	oral	other	mam.	oral	other
group	**sum**	**0.00**	**0.64**	**0.00**	**0.00**	**0.69**	**0.00**
Cephalosporines	Cefquinome	0	0	0	0	0	0
	Ceftiofur	0	0	0	0	0	0
	Cefapirin	0	0	0	0	0	0
	Cefoperazone	0	0	0	0	0	0
	Cefalexin	0	0	0	0	0	0
		0	0	0	0	0	0
Penicillin	Benzylpenicillin	0	0	0	0	0	0
	Ampicillin	0	0.01	0	0	0.02	0
	Amoxicillin	0	0.06	0	0	0.13	0
	Cloxacillin	0	0	0	0	0	0
	Fenoxymethylpenicillin	0	0.02	0	0	0.02	0
		0	0.09	0	0	0.17	0
Macrolides	Erythromycin	0	0	0	0	0	0
	Tylosin	0	0.07	0	0	0.11	0
	Tilmicosin	0	0	0	0	0	0
	Tulathromycin	0	0	0	0	0	0
		0	0.07	0	0	0.11	0
Quinolones	Danofloxacin	0	0	0	0	0	0
	Enrofloxacin	0	0	0	0	0	0
	Flumequine	0	0.09	0	0	0.07	0
	Difloxacin	0	0	0	0	0	0
	Marbofloxacin	0	0	0	0	0	0
		0	0.09	0	0	0.07	0
Sulfanomides and trimethoprim	Trimethoprim-sulfachloorpyridazin	0	0.07	0	0	0.05	0
	Trimethoprim-sulfadiazin	0	0	0	0	0	0
	Trimethoprim-sulfadoxin	0	0	0	0	0	0
	Trimethoprim-sulfamethoxazole	0	0.05	0	0	0.05	0
	Sulfadimidine	0	0.08	0	0	0.05	0
	Sulfaclozine Na	0	0	0	0	0	0
	Sulfaquinoxalin	0	0.01	0	0	0	0
		0	0.21	0	0	0.15	0
Tetracyclines	Tetracycline	0	0	0	0	0	0
	Chloortetracycline	0	0	0	0	0	0
	Doxycycline	0	0.04	0	0	0.08	0
	Oxytetracycline	0	0.1	0	0	0.07	0
		0	0.14	0	0	0.15	0
Aminoglycocides	Gentamicin	0	0	0	0	0	0
	Neomycin	0	0.02	0	0	0.03	0
		0	0.02	0	0	0.03	0
Combinations	Amoxicillin-clavulanic acid	0	0	0	0	0	0
	Amoxicillin-colistin	0	0	0	0	0	0
	Ampicillin-colistin	0	0	0	0	0	0
	Ampicillin-cloxacillin	0	0	0	0	0	0
	Dihydrostreptomycin-benzylpenicillin	0	0	0	0	0	0
	Dihydrostreptomycin-benzylpenicillin-nafcilline	0	0	0	0	0	0
	Lincomycin-neomycin	0	0	0	0	0	0
	Lincomycin-spectinomycin	0	0.02	0	0	0.01	0
	Neomycin-benzylpenicillin	0	0	0	0	0	0
	Dihydrostreptomycin	0	0	0	0	0	0
		0	0.02	0	0	0.01	0
Others	Florfenicol	0	0	0	0	0	0
	Lincomycin	0	0	0	0	0	0
	Colistin	0	0	0	0	0	0
	Tiamulin	0	0	0	0	0	0
	Pirlimycin	0	0	0	0	0	0
		0	0	0	0	0	0

Annex 3 Number of farms and number of animals in the sample

Table B3.1 lists the number of farms taking part each year and the associated number of animals.

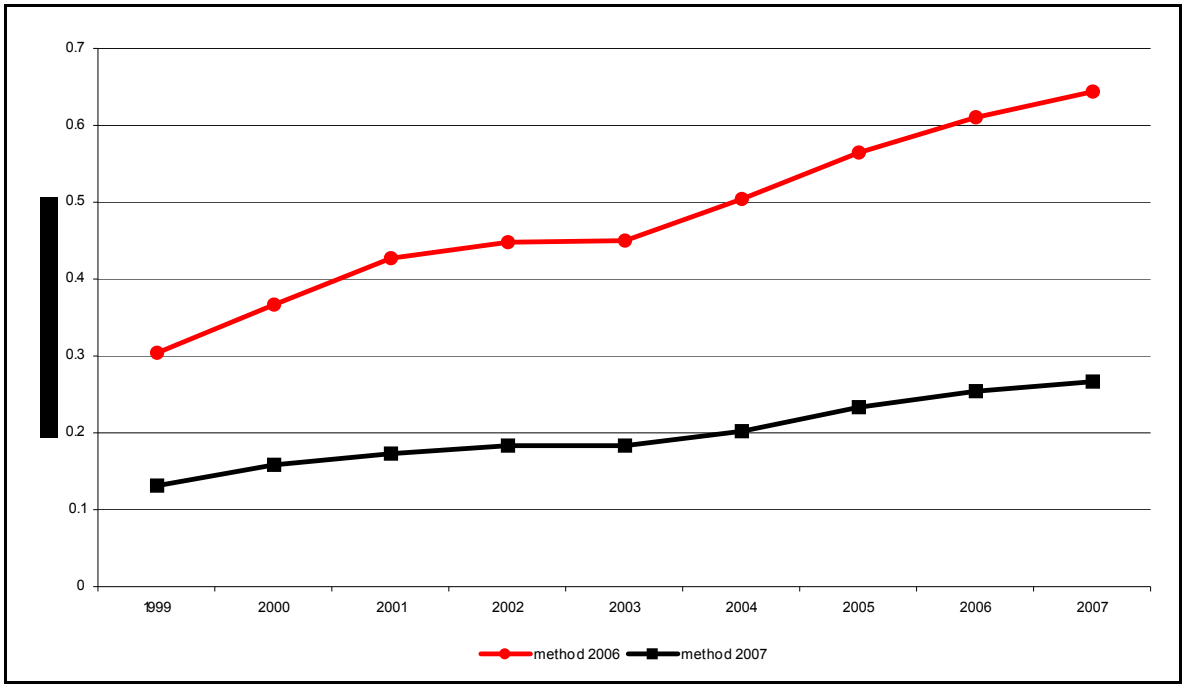
	Type of holding	2004	2005	2006	2007
No. of animals	Dairy cattle	3,919	2,962	3,099	3,025
	Sows/piglets1)	17,618	16,790	13,642	19,862
	Fattening pigs	63,740	58,622	61,503	128,807
	Broilers2)	870	1,962	2,047	1,931
Number of farms	Dairy cattle	45	36	37	36
	Sows/piglets	49	46	34	42
	Fattening pigs	39	42	33	52
	Broilers	15	29	29	29

1) These are all farms with sows and piglets, both specialised sow farms and closed pig farms**.** The sows and piglets on the closed farms are analysed with the sows/piglets and the fattening pigs on these farms with the fattening pigs**.** The specified numbers of animals are sows**.**
2) x 1,000

Annex 4 Notes to the method used to calculate the mg antibiotic use / kg body weight

Last year, a slightly different method was used for this calculation, and consequently the absolute figures cannot be compared with each other. See the following figure.
Last year the numbers of animals were obtained from LEI /CBS, agricultural and horticultural figures**.** This information has now been sourced from Eurostat to provide for comparisons between countries**.** Cattle (exclusive of calves) and sheep have now been included in the calculations for the purposes of a realistic comparison with countries with relatively large numbers of cattle and sheep.

Figure, Annex 4 the differences in the results calculated using method 2006 and method 2007



Both curves in the above figure show an increase. The red curve is considerably higher the calculations were based on the administration of the same quantity of antibiotics to a much smaller number of livestock with a lower kg body weight (i.e. exclusive of cattle and sheep).

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