# MARAN 2005

## Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands In 2005

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And Antibiotic Usage in Animals in the Netherlands
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# Colophon

This report is published under the acronym *MARAN-2005* by VANTURES, the Veterinary Antibiotic Usage and Resistance Surveillance Working Group. The information presented in *MARAN-2005* 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, Conclusions and Recommendations

## Trends in antibiotic usage

In 2005 the total sales of antibiotics for therapeutic purposes in the Netherlands increased by 55.000 kg (12%) to 508.000 kg. Total live weight production of the most important users (pigs, broilers and veal calves) increased in this period slightly by 1.1 %. As from 1998 till 2005 the total sales of antibiotics for therapeutic use has increased with 182.000 kg, every year sales have grown faster than the production of animals. In 2005 the estimated sales of antimicrobial growth promoters have declined by 35.000 kg from 75.000 to 40.000 kg.

In 2005 and in general over the last decade, sales of antibiotics for therapeutic use have increased much more than the total weight of production from livestock, whereas sales of antimicrobial growth promoters have gradually decreased. As the relative contribution for each therapeutic group remained practically unchanged, potency differences of molecules can only account for a small part of the growth in antibiotic consumption. This could for example occur when doxycycline would be replaced by tetracycline. The result would be that more kilograms of active ingredient would be necessary to medicate the same number of animals.
The data presented here confirm that the quantity and intensity of usage of antibiotics is increasing. This was also concluded in earlier MARAN-reports. Sales of quinolones and macrolides (two classes of antibiotics of which the usage in food animals is under debate because of potential public health risks) have again shown a substantial rise in 2005.

The continuous monitoring program of the Agricultural Economics Research Institute (LEI) based on farm data showed an increase in numbers of daily dosages per animal year (dd/ay) in pigs and broilers in 2005. To young piglets (and sows) most often antibiotics were administered, with broilers at second and slaughter pigs at third place. The increase in daily dosages was 6,3% in sows/piglets, 10,1% in slaughter pigs and 3,7% in broilers. Only in dairy cattle the use of antibiotics has decreased (-6,8%). Data on usage of antimicrobials in veal calves are currently lacking.

An explanation used in earlier years to justify the growth of the antibiotic sales was the emerging of new infectious diseases in pigs (PIA and circo-virus). However the presence of these diseases does not explain this year’s rise in antibiotic sales. Other causes have to be considered as well. As in other countries, in the Netherlands there are little economic incentives for restricted antibiotic usage. On the contrary, high usage of antibiotics may be rewarded with sales (industry, wholesaler and veterinarian) or with better economic results (farmer). As antibiotics are cheap, investments in housing and preventive measures may be discouraged. Furthermore, over the use of antibiotics no justification has to be made to the authorities and the general public.

The Dutch professional association for veterinarians (KNMvD) has an active antibiotic policy to promote restrictive and selective use of antibiotics. The continuous rise in sales of antibiotics demonstrates that this goal is not achieved. Obviously, self-regulation in this competitive market is failing. At the moment Directive 2004/28/EU on the community code relating to veterinary medicinal products is implemented in Dutch law. This process could be used to implement measures that stimulate more selective and restrictive use of antibiotics.

**Trends in resistance**

*Breakpoints*

In 2005 for the first year epidemiological cut-off values for the wild-type distributions were used for the MIC-data analysis for the food-borne pathogens and indicator organisms, in stead of the previously used clinical breakpoints (e.g. CLSI). The reason is the using cut-off values will result in more sensitive detection of acquired resistance in these organisms. This may result in a change in resistance percentages in comparison with previous years. For trends analysis these cut-off values were also used on data sets from previous years.

*Salmonella*

Resistance levels in *S.* Enteritidis showed a dramatic change in Phage Type (PT) 4 from Dutch layers. For the first year a high percentage of quinolone resistance was observed in PT4 from Dutch layers. This was most probably related to import of resistant organisms and not by selection through usage of (fluoro)quinolones in these animals. Quinolone resistance in *S.* Enteritidis from human patients was predominantly related to PT1 and to a lesser extent to PT4. This indicates that quinolone resistant strains of *S.* Enteritidis isolated from humans predominantly originate from imported animal products or from travel related infections.

High level resistance to ciprofloxacin was only incidentally detected in *S.* Kentucky strains isolated from human patients (also detected in 2002, 2003 and 2004). These strains were related to travel to North African countries and not to Dutch food-animals.

Striking is the increase in resistance to modern third-generation cephalosporins in salmonella's from poultry. This is most likely caused by transfer of resistance plasmids from commensal *E. coli* in these animals.

*Campylobacter* spp.

In 2005 for the first year *Campylobacter* spp. from dairy cows and veal calves were included in the surveillance. Isolates from veal calves showed the highest levels of resistance and multi drug resistance, whereas isolates from dairy cows were mostly susceptible.

Also for the first year isolates from poultry raw meat products imported from Southern America and from biologically reared poultry and pigs were included. Resistance to erythromycin, representing the first choice drug for human therapy of campylobacteriosis, occurred more frequently in isolates from imported products, than in isolates from Dutch food animals. Surprisingly in isolates from biological poultry and pigs resistance levels were similar to those of conventionally reared animals. Colonisation of biological animals with resistant campylobacters from the environment may be an explanation.

Both in isolates from humans and food animals the resistance levels showed a general tendency to increase, except erythromycin resistance in *C. coli* from pigs which decreased after the ban of the growth promoter tylosine in 1999.

*E. coli*

The resistance levels for *E. coli* O157 were low and limited to a small number of individual isolates. Two isolates from cattle were multi-drug resistant. In 2005 an outbreak occurred with a cluster of 25 human cases. This outbreak clone was resistant to trimethoprim and sulphamethoxazole.

In *E. coli* strains isolated from faeces (indicator organisms for the commensal gut flora) from intensively reared broilers and veal calves, and to a slightly lesser extend slaughter pigs, resistance levels are very high and show a tendency to increase over time. In dairy cows resistance is only rarely present.

Multi drug resistance shows a similar increasing trend, with alarmingly high levels of multi drug resistant isolates in broilers and veal calves.

The occurrence of extended spectrum beta-lactamases (ESBLs) increased substantially in broilers from 9.7% in 2004 to 14.1% in 2005, this in spite of the fact that cephalosporins are not used in these animals. This means that linkage of resistance genes and co-selection by usage of other antibiotics probably has been the main cause for the observed increase.

Resistance levels in isolates from imported poultry products from Southern America were higher while resistance in isolates from biological animals was lower compared to those from Dutch meat products or conventional Dutch animals.

*Enterococcus* spp.
In isolates from dairy cows the lowest resistance levels were observed as was expected. In these animals the main usage of antibiotics is in local treatment of mastitis or intra-uterine infections. In the major food producing animals (broilers, slaughter pigs and veal calves) resistance occurred more frequently.
Amoxicillin resistance was only observed at relatively low levels in *E. faecium* isolated from cattle and broilers. In veal calves amoxicillin resistance substantially increased from 0% in 1997 to 10.7% in 2005, in broilers the levels were stable.
For the first year a few linezolid resistant strains were detected in veal calves and in poultry products, this in spite of the fact linezolid, a member of the oxazolidone class, is not used in animals at all.
In 2005 high level ciprofloxacin resistant *E. faecalis* isolates were observed (MIC $\geq$ 16 mg/L). These strains were isolated from both veal calves and broilers, the animal species in which quinolones are predominantly used.

Methicillin resistant *S. aureus* (MSRA)
In 2005 and 2006 a widespread clonal distribution of MRSA Sequence Type 398 was discovered in food animals in the Netherlands. The prevalence of carriers in pigs was very high, 39% of all animals examined at slaughterhouses and 81% of the slaughter batches of pigs examined were positive for this clone. A small study in pig farmers revealed that 23% were carrying the clone in their noses, compared to 0.03% prevalence in the open population. Studies in faeces of food animals and recently published data indicate a widespread occurrence of the clone in different food animal species and countries. Infection prevention measures for MRSA have been adjusted for patients at risk admitted to hospitals, and a large research project has been initiated in the Netherlands by a combined effort of the Ministry of Public Health Welfare and Sport and the Ministry of Agriculture, Nature and Food Quality.

Animal pathogens *Mannheimia haemolytica, Pasteurella multocida*
Resistance to tetracycline occurred most frequently in both species, but, as was observed for amoxicillin, predominantly in *M. haemolytica* (MHA).
The resistance level of enrofloxacin was somewhat misleading in MHA, 2.5% were classified resistant, but another 20% showed reduced susceptibility, indicating the presence of acquired resistance. Resistance to ceftiofur, tilmicosin and florfenicol was not detected.
Strains isolated from veal calves were for all antibiotics tested more resistant than those from dairy cattle, reflecting the difference in use practices of antibiotics in these animal husbandry systems.

Mastitis pathogens *E. coli*, coliform bacteria, *Staphylococcus aureus*, coagulase negative staphylococci, *Streptococcus uberis* and *S. dysgalactiae*
In general *E. coli* strains isolated from milk samples from cows suffering from mastitis were susceptible to most antibiotic classes. Only resistance to the older antibiotics (amoxicillin, tetracycline, streptomycin and trim/sulpha) occurred more frequently than in single isolates.
The coliform bacteria (a.o. *Klebsiella*, *Enterobacter*, *Citrobacter*) showed a high level of resistance to amoxicillin and to the combination with clavulanic acid and cefuroxime. All isolates were susceptible to cefoperazone and cefquinome.
The *S. aureus* isolates tested were susceptible to most antibiotics, but 7.1% were penicillin resistant. Oxacillin resistance (MRSA) was not present. The coagulase negative staphylococci were more resistant than *S. aureus*, 61.1% were resistant to penicillin and 5.2% to oxacillin (*mec*A-positive).
Based on the CLSI criteria in the streptococci only resistance to erythromycin, lincomycin, pirlimycin and tetracycline was observed. However, more than 30% of *S. uberis* showed reduced susceptibility to penicillin. In 2005 *S. uberis* was more frequently resistant to erythromycin, lincomycin and pirlimycin than *S. dysgalactiae*. Resistance to tetracycline was highest in *S. dysgalactiae*.

**Conclusions and recommendations**
It can be concluded that therapeutic usage of antibiotics in food animals in The Netherlands substantially increased again in 2005. Although data in this report show an increase in antibiotic usage

at farm level in pigs and poultry, this increase does not fully explain the rise in antibiotics sales data. The difference in usage in daily dosages versus the usage in grammes is of course an important factor here, but maybe also the lack of data about the use of antibiotics in veal calves.

The resistance levels in animal bacteria show a simultaneous tendency to increase. A sudden appearance of resistance to quinolones was observed in *S.* Enteritidis isolated from Dutch layers not found in earlier years. Moreover, the occurrence of ESBL-producing isolates clearly increased in broilers.

The frequent occurrence and increase in levels of multi drug resistance (MDR) in strains isolated from the gut of food animals (broilers, veal calves and to a slightly lesser extend also pigs) is a worrying trend. This trend reflects the intensive use of antibiotics in Dutch food animals, by which an environment is created, which provides advantages for MDR strains. The occurrence of methicillin resistant *Staphylococcus aureus* (MRSA) in Dutch pigs can be interpreted as an evolutionary consequence of frequent and repetitive selection pressure by antibiotic usage in food animals.

The observed trends in usage and resistance development warrant an evaluation of the existing practice of prescribing of antibiotics in food animals. An effective incentive-structure has to be developed, both for farmers and for other actors in the supply chain. Requirements by buyers can play an important role in this respect. Possibilities for increased registration of usage and control measures are currently under debate. At the moment Directive 2004/28/EU on the community code relating to veterinary medicinal products is implemented in Dutch law. This process could be used to pinpoint consumption and to implement measures that stimulate more selective and restrictive use of antibiotics.

Data in this report show that in general resistance levels in biologically reared food animals were lower than the levels in conventional food animals. Moreover, resistance in imported poultry products was common and showed different patterns than in Dutch poultry.

Conventional food animal production of broilers and veal calves and to a lesser extend pigs can be considered risk factors for frequent presence of antimicrobial resistance. In broilers the short life, mass medication and frequent repopulation of houses may be important determinants. In veal calves the fact that all animals on a farm originate from different dairy farms, including many foreign sources will lead to a continuous introduction of new organisms and resistance genes. Selection pressure in the veal calf industry by flock administration is high as well.

Based on the data in this report it can be recommended that:

- Measures to improve the quality of registration of drug usage and control strategies for over-usage should be implemented in the Netherlands
- The control of imported food products for resistant organisms should be continued and expanded
- Further research aimed at the causes for the constant increase of the use of antibiotics in needed, including identification of risk factors.
- The occurrence and trends in multi drug resistance (including ESBLs and quinolone resistance) should be studied in detail. This includes studies aimed at:
    - determinants that select for MDR
    - epidemiology of MDR at farms
    - molecular epidemiology of MDR isolates
    - dynamics of MDR genes and/or strains under different use conditions

# Samenvatting, Conclusies en Aanbevelingen

**Gebruik van antibiotica**
In 2005 zijn de verkoopcijfers van antibiotica voor therapeutisch gebruik in dieren met 55.000 kg gestegen (12%) tot 508.000 kg. De productie van levend gewicht van de belangrijkste diersoorten die antibiotica krijgen toegediend (varkens, vleeskuiken en vleeskalveren), nam in deze periode toe met 1.1%. Van 1998 tot 2005 is het totale therapeutische gebruik met 182.000 kg sneller gestegen dan de dierlijke productie Nederland. In 2005 is het geschatte gebruik van groeibevorderaars met 35.000 kg afgenomen van 75.000 naar 40.000 kg..

In 2005, maar ook in het decennium daarvoor, zijn de verkoopcijfers van antibiotica voor therapeutisch gebruik in dieren veel sterker toegenomen dan de aantallen landbouwhuisdieren. Dit terwijl het gebruik van groeibevorderaars langzaam is afgenomen. Omdat de relatieve bijdrage van iedere klasse van antibiotica aan het totaal grofweg gelijk is gebleven, kunnen potentieverschillen van gebruikte werkzame stoffen deze toename slechts voor een klein deel verklaren. Dit zou b.v. een rol kunnen spelen als doxycycline vervangen wordt door tetracycline. Dan zijn er meer kilo's werkzame stof nodig om een zelfde aantal dieren te medicineren.
De gegevens in dit rapport bevestigen dat antibiotica steeds meer en intensiever worden gebruikt. Dit is ook in eerdere MARAN-rapporten geconcludeerd. De verkopen van quinolonen en macroliden (twee klassen van antibiotica waarvan het gebruik bij landbouwhuisdieren ter discussie staat wegens potentiële risico's voor de volksgezondheid) zijn in 2005 opnieuw substantieel toegenomen.

Het monitoringsprogramma van het Landbouw Economisch Instituut (LEI) op basis van boekhoudkundige bedrijfsgegevens van antibioticumgebruik in kippen, varkens en runderen laat een toename zien in aantallen dagdoseringen per dierjaar (dd/ay) in varkens en vleeskuikens. Aan jonge biggen (en zeugen) wordt het vaakst een antibioticum toegediend, met vleeskuikens op de tweede en vleesvarkens op de derde plaats. De aantallen dagdoseringen per dierjaar zijn bij zeugen/biggen met 6,3% toegenomen, bij vleesvarkens met 10,1% en bij vleeskuikens met 3,7%. Alleen bij melkvee is sprake van een daling van het gebruik (- 6,8%). Informatie over gebruik bij vleeskalveren ontbreekt.

In voorgaande jaren werd het opkomen van nieuwe infectieziekten in varkens (PIA en Circo-virussen) als mogelijke verklaring gebruikt. Dit kan de toename in 2005 niet verklaren, er lijken andere oorzaken aan ten grondslag te liggen. Net als in andere landen bestaan er te weinig economische prikkels die restrictief gebruik van antibiotica stimuleren. Het veelvuldige gebruik van antibiotica heeft juist een economisch voordeel voor betrokken partijen (industrie, groothandel, dierenarts) en het leidt tot betere economische resultaten voor de veehouder. Omdat antibiotica goedkoop zijn worden investeringen gericht op verbetering van de huisvesting en is er minder aandacht voor preventieve maatregelen. Verder hoeft er geen verantwoording te worden afgelegd over antibioticagebruik aan de overheid of aan publieke partijen.

De Koninklijke Nederlandse Maatschappij voor Diergeneeskunde heeft sinds 1994 een actief antibioticumbeleid geformuleerd wat gericht is op het promoten van een selectief en restrictief gebruik van antibiotica in dieren. De continue toename in verkoopcijfers van antibiotica maken duidelijk dat de doestellingen van dit beleid niet zijn gehaald. Een zelf-regulerend mechanisme in deze competitieve markt ontbreekt kennelijk.
Momenteel wordt Richtlijn 2004/28/EU aangaande de EU-Code voor Veterinaire Medicinale Producten geïmplementeerd in de Nederlandse wetgeving. Dit proces kan worden gebruik om maatregelen in te voeren die leiden tot selectiever en restrictiever gebruik van antibiotica in landbouwhuisdieren.

**Trends in Resistentie**

*Breekpunten*
Voor de analyse voor de gegevens van de voedselpathogenen en indicatororganismen uit 2005 werd voor de eerste keer de zogenaamde epidemiologische cut-off waarden van de wild-type verdelingen gebruikt in plaats van klinisch gericht breekpunten (b.v. CLSI), die in voorgaande jaren werden gebruikt. De reden is dat het gebruik van cut-off waarden leidt tot een verbetering van de gevoeligheid van het detecteren van verkregen resistentie. Dit heeft tot gevolg dat resistentiepercentages kunnen zijn veranderd in vergelijking met voorgaande jaren. Voor de trendanalyses zijn deze cut-off waarden ook toegepast op de oudere data uit eerdere jaren.

*Salmonella*
In 2005 werd een sterke toename gezien in quinolonen resistentie in *S.* Enteritidis faagtype 4 uit Nederlandse legkippen. Dit is waarschijnlijk veroorzaakt door importen van gecontamineerde broedeieren of kuikens en niet door gebruik van (fluoro)quinolonen in deze dieren. Quinolonen resistentie in *S.* Enteritidis in patiënten werd voornamelijk gevonden in faagtype1 en in mindere mate in faagtype 4. Dit wijst er op dat de infecties in deze patiënten te herleiden zijn tot consumptie van besmette geïmporteerde eieren of werd opgedaan tijdens reizen in het buitenland.
Hoge ciprofloxacin-resistentie kwam slechts incidenteel voor in *S.* Kentucky geïsoleerd uit patiënten. Dit resistente serotype is jaarlijks bij de mens waargenomen sinds 2002. De infecties waren gerelateerd aan reizen naar Noord-Afrikaanse landen en hadden geen Nederlandse (dierlijke) bron.
Zeer opvallend is de toename in resistentie tegen moderne cefalosporinen (ESBL's) in salmonella's uit pluimvee. Dit wijst op overdracht van ESBL's door middel van plasmiden vanuit commensale *E. coli* in deze dieren.

*Campylobacter* spp.
In 2005 werden voor het eerste jaar *Campylobacter* spp. uit melkkoeien en vleeskalveren opgenomen in de surveillance. Isolaten uit vleeskalveren vertoonden de hoogste resistentie waarden en multiresistentie, terwijl de isolaten uit melkvee meestal volledig gevoelig waren.
Ook werden voor de eerste keer isolaten uit pluimveevlees, geïmporteerd uit Zuid-Amerika, onderzocht. In deze stammen kwam resistentie tegen erythromycine, het eerste keuze-middel bij de behandeling van campylobacteriose bij de mens, beduidend vaker voor dan in stammen uit Nederlandse landbouwhuisdieren. Wat voorts opviel was dat stammen uit biologisch gehouden kippen en varkens vergelijkbare resistentie niveaus vertoonden als die uit conventioneel gehouden dieren. Een verklaring kan zijn dat de biologisch gehouden dieren gekoloniseerd zijn met stammen uit de omgeving die hun oorsprong hebben in de intensieve veehouderij.
Zowel in stammen uit dieren als in die van de mens werd een toename in resistentie waargenomen, behalve voor erythromycine-resistentie in *C. coli* uit varkens. Na het verbod op het gebruik van tylosine als groeibevorderaar in 1999 nam het resistentieniveau daarvan af.

*E. coli*
In *E. coli* O157 werd weinig resistentie gevonden en indien aanwezig was dit beperkt tot een klein aantal individuele isolaten. Twee stammen uit runderen waren multiresistent. In 2005 was er een uitbraak met een cluster van 25 humane infecties. Deze uitbraakstam was resistent tegen trimethoprim en sulfamethoxazole.
In *E. coli* als indicatororganisme voor de commensale darmflora van vleeskuikens en vleesvarkens en in iets mindere mate ook van vleesvarkens, zijn de resistentieniveau's erg hoog. Bovendien vertonen deze een stijgende trend. In stammen uit melkkoeien komt resistentie slecht zelden voor.
De aanwezigheid van multiresistentie neemt ook duidelijk toe sinds 1998, met alarmerend hoge frequenties van multiresistente stammen in vleeskuikens en vleeskalveren.
In vleeskuikens zette zich in 2005 de al in 2004 waargenomen toename in resistentie tegen de moderne cefalosporinen voort (9.7% in 2004 naar 14.1% in 2005). Omdat cefalosporinen in vleeskuikens niet worden gebruikt is de enige verklaring voor dit fenomeen dat er koppeling is van deze ESBL's aan andere resistentiegenen en dat co-selectie door gebruik van andere antibiotica bepalend is geweest.

In isolaten uit pluimveeproducten uit Zuid-Amerika werd meer resistentie gevonden en in stammen uit biologisch gehouden dieren minder resistentie dan in stammen uit dierlijke producten of dieren uit de Nederlandse intensieve veehouderij.

*Enterococcus* spp.
Zoals verwacht waren de stammen uit melkvee het minst resistent. In deze dieren beperkt de bulk van het antibioticumgebruik zich tot behandeling van mastitis of intra-uteriene infecties. In de belangrijkste voedsel producerende dieren (vleesvarkens, vleeskalveren, vleeskuikens) werd meer resistentie waargenomen.
Amoxicilline resistentie werd alleen gezien in *E. faecium* uit runderen en pluimvee. In vleeskalveren werd hiervan een duidelijke toename gezien van 0% in 1997 tot 10.7% in 2005, in vleeskuikens was het niveau stabiel.
In 2005 werden voor het eerst linezolid resistente stammen gezien in vleeskalveren en pluimvee producten, dit ondanks het feit dat oxazolidones niet in dieren worden gebruikt.
In 2005 werden hoog ciprofloxacin resistente stammen gezien (MIC $\geq$ 16 mg/L) in vleeskalveren en vleeskuikens, de species waar quinolonen het meest gebruikt worden.

Methicilline-resistente S. aureus (MSRA)
In 2005 en 2006 werd een wijdverspreide klonale distributie van MRSA Sequence Type 398 ontdekt in voedselproducerende dieren in Nederland. De prevalentie van dragers in varkens was erg hoog, 39% van alle onderzochte dieren op slachthuizen en 81% van de onderzochte slachtbatches varkens waren positief voor deze kloon. Een kleine studie in varkenshouders toonde aan dat 23% drager was van deze MRSA in de neus, vergeleken met 0.03% dragerschap in de open populatie. Onderzoek in mest van voedselproducerende dieren en recente publicaties maken aannemelijk dat de kloon wijdverspreid in verschillende dieren en Europese landen voorkomt.
Infectieziekten preventieve maatregelen gericht op MRSA bij opname in een ziekenhuis van patiënten at risk zijn aangescherpt. Als reactie op deze bevindingen is in Nederland een groot onderzoeksproject gestart geïnitieerd door het Ministerie van landbouw, Natuurbeheer en Voedselkwaliteit in samenwerking met het Ministerie voor Volksgezondheid, Welzijn en sport.

Dierpathogenen *Mannheimia haemolytica, Pasteurella multocida*
Tetracycline resistentie kwam het meest voor in beide species, zoals ook werd gezien voor amoxicilline, maar vooral in *M. haemolytica* (MHA).
Het resistentieniveau voor enrofloxacin was ietwat misleidend in MHA. Tweeëneenhalf procent werden als resistent geclassificeerd, maar nog eens 20% vertoonden een duidelijke afname in gevoeligheid, als gevolg van selectiedruk door gebruik van dit middel. Resistentie tegen ceftiofur, tilmicosin en florfenicol werd niet gevonden.
Isolaten uit vleeskalveren waren voor alle antibiotica meer resistent dan die uit melkkoeien, wat een afspiegeling is van het verschil in antibioticumgebruik in beide houderijsystemen.

Mastitispathogenen *E. coli*, coliform bacteriën, *Staphylococcus aureus*, coagulase negatieve stafylokokken, *Streptococcus uberis* and *S. dysgalactiae*
*E. coli* geïsoleerd uit melk van koeien met mastitis was meestal gevoelig voor de onderzochte antibiotica. Enkel resistentie tegen de oudere middelen (amoxicilline, tetracycline, streptomycine en trim/sulfa) kwam wat vaker voor. De coliforme bacteriën (waaronder *Klebsiella, Enterobacter, Citrobacter*) waren echter meestal resistent tegen amoxicilline, de combinatie met clavulaanzuur en cefuroxime. Alle isolaten waren nog wel gevoelig voor de 3$^{e}$ en 4$^{e}$ generatie cefalosporinen cefaperazone en cefquinome.
*S. aureus* was meestal gevoelig voor de geteste antibiotica, maar 7.1% was penicilline resistent. Oxacilline resistentie (MRSA) werd in *S. aureus* niet gevonden. In coagulase negatieve stafylokokken werd meer resistentie gezien, 61.1% was resistent tegen penicilline, waarvan 5.2% ook oxacilline resistent was door aanwezigheid van het *mec*A-gen.
Op basis van de door de CLSI voorgeschreven interpretatiecriteria werd in de onderzochte streptokokken alleen resistentie tegen erythromycine, lincomycine, pirlymycine en tetracycline gevonden. Echter meer dan 30% van de *S. uberis* stammen vertoonde verminderde gevoeligheid voor

penicilline. In 2005 werd in *S. uberis* meer resistentie tegen erythromycine, lincomycine en pirlimycine gezien dan in *S. dysgalactiae*. Tetracycline resistentie kwam echter het vaakst voor in *S. dysgalactiae.*

**Conclusies en aanbevelingen**
Er kan worden geconcludeerd dat in Nederland het therapeutische gebruik van antibiotica beduidend toenam in 2005. Op boerderijniveau werd ook een toename in antibioticumgebruik gezien bij zowel varkens als vleeskuikens, echter deze toename kan de gerapporteerde toename in verkoopcijfers van de FIDIN niet geheel verklaren. Het verschil tussen gebruik in dagdoseringen en in grammen speelt hierbij uiteraard een belangrijke rol, maar mogelijk ook het ontbreken van gebruikscijfers over de vleeskalveren.

Niet alleen het gebruik van antibiotica maar ook de resistentieniveaus in de in landbouwhuisdieren onderzochte bacteriën vertonen een toenemende trend. Zeer opvallend was het plotselinge frequente voorkomen van quinolonen-resistentie in *S.* Enteritidis in 2005, iets wat in voorgaande jaren niet werd gezien. Daarnaast werd een opvallende toename gezien in het voorkomen van ESBL-positieve *E. coli* en *Salmonella* stammen in vleeskuikens. Het frequente voorkomen en ook de toename in multiresistente (MDR) stammen in het maagdarmkanaal van voedselproducerende dieren (vleeskuikens, vleeskalveren en iets mindere mate ook vleesvarkens) is een zorgwekkende trend. Dit is een afspiegeling van het intensieve gebruik van antibiotica in die dieren waardoor een milieu is ontstaan waarin MDR isolaten zich goed kunnen handhaven. De recente ontdekking van het op grote schaal voorkomen van methicilline-resistente *S. aureus* (MRSA) in Nederlandse vleesvarkens dient te worden beschouwd als een evolutionaire consequentie van de frequente en telkens herhaalde selectiedruk door gebruik van antibiotica in deze dieren.

De waargenomen trends in gebruik en resistentie zijn een signaal voor een evaluatie van de huidige wijze waarop antibiotica worden voorgeschreven in voedselproducerende dieren. Er zal een effectieve incentive-structuur moeten worden ontwikkeld, zowel direct richting veehouder als voor andere schakels in de productiekolom. Eisen door afnemers kunnen daarbij een belangrijke rol spelen. Mogelijkheden voor een betere registratie van het gebruik en betere controlemaatregelen worden momenteel in Nederland bediscussieerd. De implementatie van Richtlijn 2004/28/EU aangaande de EU-Code voor Diergeneesmiddelen in Nederland kan instrumenteel zijn voor het stimuleren van maatregelen gericht op selectiever en restrictiever gebruik van antibiotica.

Gegevens in dit rapport laten zien dat in biologisch gehouden dieren in het algemeen minder resistentie voorkomt dan in dieren uit de intensieve veehouderij. Daarnaast werd meer resistentie gevonden in stammen uit Zuid Amerika geïmporteerde pluimveeproducten.

De intensieve vleeskuiken, vleeskalver- en in mindere mate ook de vleesvarkenshouderij zijn te beschouwen als risicofactoren voor het frequente voorkomen van antibioticumresistente organismen. In vleeskuikens zijn de korte levensduur, selectiedruk door koppelmedicatie en de frequente herbevolking van stallen mogelijke bepalende factoren. In vleeskalveren speelt een rol dat alle dieren op een bedrijf van een ander melkveebedrijf afkomstig zijn en dat in potentie op ieder bedrijf ook kalveren uit het buitenland aanwezig zijn. Dit heeft tot gevolg dat er een continue instroom is van nieuwe organismen en nieuwe resistentiegenen in deze bedrijven. Selectiedruk door frequente koppelbehandelingen in vleeskalveren zorgt voor uitselectie en verspreiding van resistente organismen en resistentiegenen.

Gebaseerd op de data uit dit rapport kan het volgende worden aanbevolen:
- Er dienen maatregelen te worden genomen om te komen tot en betere registratie van het diergeneesmiddelengebruik in Nederland. Daarnaast verdient het aanbeveling een controlestrategie gericht op overmatig gebruik te implementeren
- Het controleren van dierlijke producten die worden geïmporteerd dient te worden gecontinueerd en uitgebreid.

- Er is nader onderzoek nodig naar de oorzaken van het steeds toenemende gebruik van antibiotica. Risicofactoren moeten worden geïdentificeerd.
- De oorzaken van de waargenomen trends in multiresistentie (inclusief ESBLs en quinolonen resistentie) dienen in meer detail te worden onderzocht. Dit omvat o.a. onderzoek naar:
  - Determinanten voor selectie van MDR-organismen
  - Epidemiologie van MDR op bedrijven
  - Moleculaire epidemiologie van MDR-organismen
  - Dynamiek van MDR-organismen en genen onder variabele condities

# I Usage of antibiotics in animal husbandry in the Netherlands

**Highlights**
In 2005 the total sales of antibiotics for therapeutic purposes in the Netherlands increased by 55.000 kg (+ 12%) to 508.000 kg. Total live weight production in the Netherlands of the food animal species to which most antibiotics are administered (pigs, broilers and veal calves) increased in this period slightly by 1.1 %. As from 1998 till 2005 the total sales of antibiotics for therapeutic use has increased with 182.000 kg, every year sales have grown faster than the production of animals. In this period the sales of antimicrobial growth promoters have declined by 35.000 kg (estimated) from75.000 to 40.000 kg.
The continuous monitoring program of the Agricultural Economics Institute (LEI) based on farm data showed an increase in numbers of daily dosages per animal year (dd/ay) in pigs and broilers in 2005. Obviously to young piglets (and sows) most often antibiotics were administered, with broilers at second and slaughter pigs at third place.

## *Usage of antimicrobial growth promoters (AGPs) and coccidiostats*

In the Netherlands, manufacturing, distributing and selling of animal feed containing (AGPs) and coccidiostats was in the hands of the feed industry and was not controlled by veterinarians. In 1998 250.000 kg of antibiotics were used as AGPs in the Netherlands. Since cross resistance occurs between antibiotics used as AGP and antibiotics used therapeutically for animals and humans, the use of antibiotics as AGP is put under pressure. Since 1999 only a few antibiotics were still allowed and used as AGP. The prohibition of the use of the remaining antibiotics as from January 2006, completed the EU drive to phase out all AGPs from livestock production. At the end of 2005 the sales of AGP's stopped given that stocks with raw materials and medicated animal feed had to be sold and used before 2006. Therefore in 2005 the sales of AGP's were already reduced to 40.000 kg, a decrease of 35.000 kg compared to 2004 (estimated after consulting manufacturers).

## *Usage of antibiotics as medicines for therapeutic purposes*

### Total sales, provided by the pharmaceutical industry

Since 1990 the therapeutic use of antibiotics in the Netherlands has been monitored, based on total sales data provided by the FIDIN (manufacturers and importers of veterinary medicines in the Netherlands). In table 1 the most recent sales data (2005) are shown. Sales from 1997 to 2005, expressed in kg, and the relative contribution of each therapeutic group are summarized in figure 1. In table 2 most recent data on numbers of Dutch livestock (2005) from the agricultural census are shown. In table 3 live weight production[1] (2005) is reported. Livestock statistics over a longer period (from 1997 to 2005) are summarized in figure 2 and figure 3.

The total sales of antibiotics increased in 2005 by 55.000 kg to 508.000 kg (+12%) (Table 1). Expressed in percentages, the sales of penicillines/cephalosporines (+24%), aminoglycosides (+22%) and macrolides (+21%) increased most rapidly.

Pigs, broilers and veal calves are known to be the food animals to which most antibiotics for therapeutic use are administered in The Netherlands. Therefore it is relevant to relate changes in antibiotic sales to demographic developments in these animal groups. According to the agricultural census in April (Statistics Netherlands, CBS) the number of pigs increased by 1,4% (table 2). On the other hand, a survey of the Product Board for Livestock, Meat and Eggs in August 2005 showed a slight reduction of the number of pigs compared to 2004 (0,9%). The live weight production of pigs however remained more or less unchanged according to PVE (table 2). The number of broilers

[1] Live weight production is calculated by correcting gross indigenous product (bruto eigen productie: BEP) with the killing out percentage. Killing out percentages used: cattle 50%, veal calves 60%, pigs 81%, poultry 74%.

increased by 0,5 % and live weight production in poultry increased by 2,5%. The veal calf population increased by 8,4%, the live weight produced by veal calves increased by 4,4%. Total live weight production from veal calves, pigs and poultry increased by 1,1% in 2005 (figure 3).

As from 1997 onwards, total sales of antibiotics for therapeutic use have increased from 332.000 kg to 508.000 kg in 2005 (+53 %) (Figure 1). This is an average increase of 6 % per year. The veal calf population over this period increased by 8,6 %, the broiler population slightly decreased by 1,1 % and the pig population decreased over this period by 25% (figure 2).

Because in 1997 live weight production was influenced by the outbreak of swine fever in the Netherlands, this is not a representative year to compare antibiotic usage and live weight production. In 1998 the live weight produced in veal calves, pigs and poultry amounted to 2.923 million kilogram. From 1998 onwards, the total live weight production of pigs, veal calves and broilers decreased by 10,8% (figure 3). Antibiotic usage per 1000 kg live weight production in 1998 was 0,094 mg, this gradually increased to 0,164 mg in 2005 (figure 4).

In general the relative contribution of different therapeutic groups of antibiotics to total sales has remained stable over the years. In 2005 tetracyclines and trimethoprim/sulphonamide combinations represented 78% of the weight of total sales in antibiotics; in 1997 both classes represented 75%.

**Table 1. Total sales of antimicrobials in 2005 in the Netherlands.**

| Therapeutic group | kg of active substance in 2005 (x1000) | Difference with 2004 |
|---|---|---|
| Penicillins/cephalosporins | 54 | 24 % |
| Tetracyclines | 307 | 14 % |
| Macrolides | 29 | 21% |
| Aminoglycosides | 11 | 22 % |
| Quinolones and fluoroquinolones | 8 | 14 % |
| Trimethoprim/sulphonamides | 93 | 0 % |
| Other | 6 | 0 % |
| Total | 508 | 12 % |

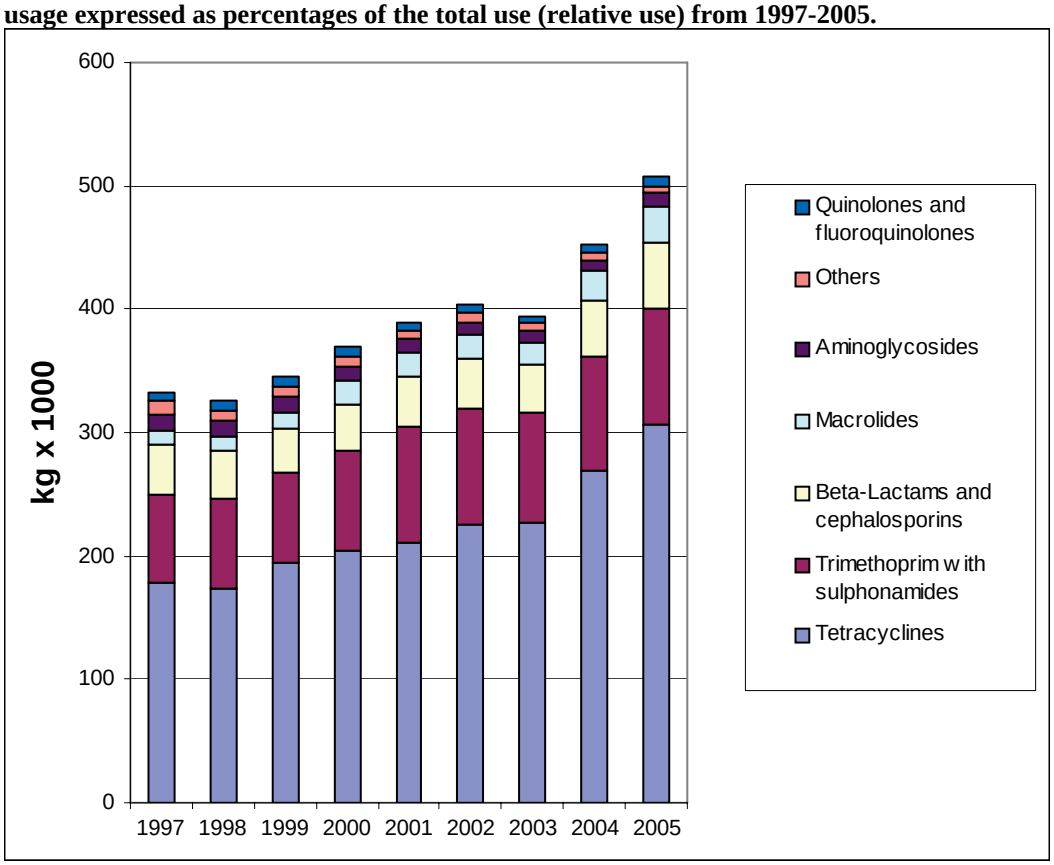
Source: FIDIN.

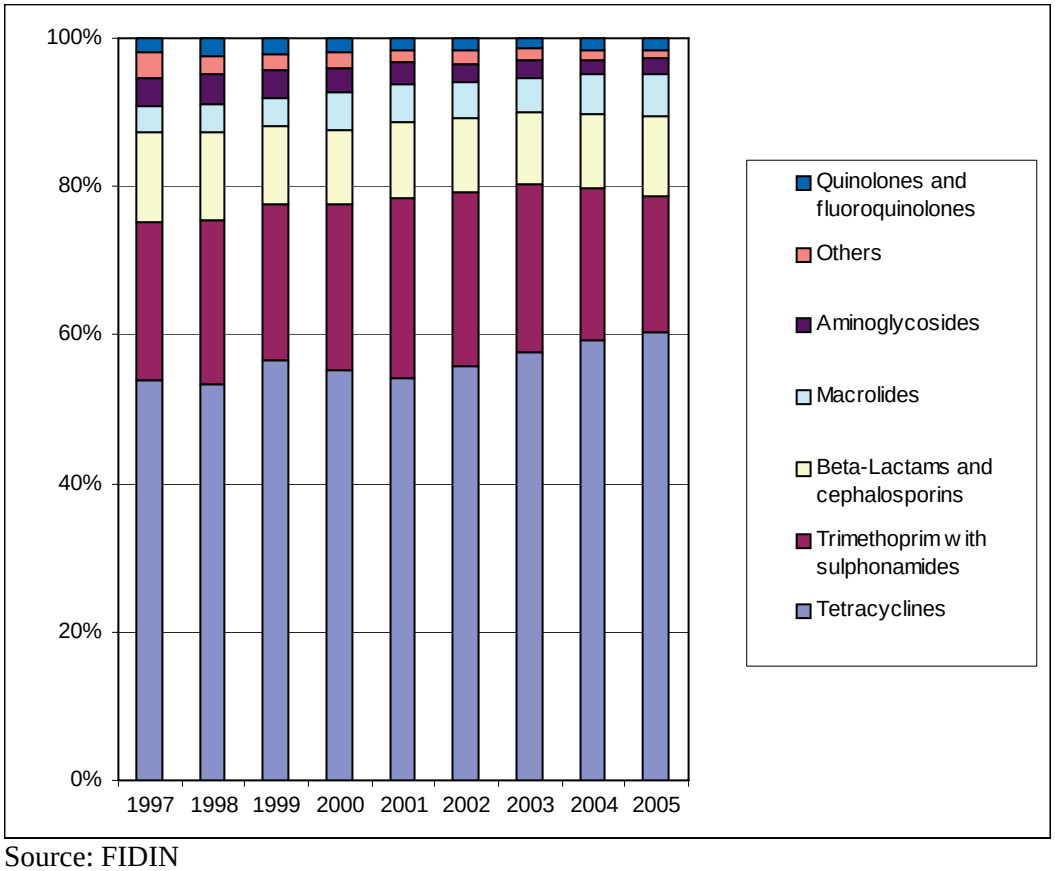
**Table 2. Agricultural census in the Netherlands (2005), numbers x 1000**

| Animal species | N x 1000 in 2005 | Difference with 2004 |
|---|---|---|
| Dairy cattle | 2.588 | -1,8 |
| Veal calves | 829 | 8,4 |
| Cows for fattening and grazing | 382 | 3,8 |
| Cattle total | 3.799 | 0,8 |
| | | |
| Pigs for fattening (>20kg) | 5.504 | 2,3 |
| Piglets | 4.563 | 0,9 |
| Pigs other | 1.244 | -0,2 |
| Pigs total | 11.311 | 1,4 |
| | | |
| Broilers | 44.496 | 0,5 |
| Laying hens (until 2004 inclusive breeding) | 41.047 | - |
| Laying hens breeding | 1.582 | - |
| Broilers, breeding | 5.788 | -1,7 |
| Ducks and Turkeys | 2.276 | 3,5 |
| Poultry total | 95.189 | 8,2 |
| | | |
| Sheep | 1.363 | 10,3 |
| Rabbits | 360 | 3,4 |
| Goats | 292 | 3,5 |
| Horses and Ponies | 133 | 3,1 |

Source: Agricultural census, Statistics Netherlands (CBS).

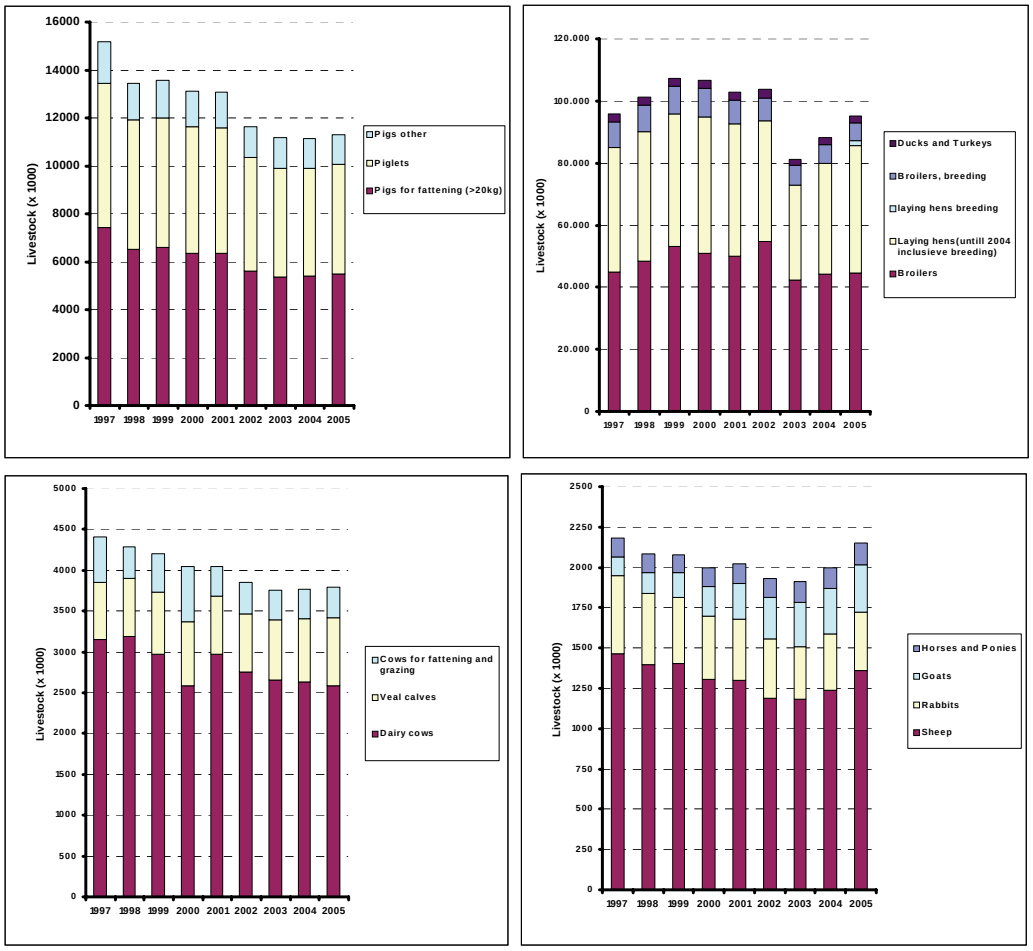
**Figure 1. Usage of antibiotics for therapeutic use (active ingredient x 1000 kg) in the Netherlands and the usage expressed as percentages of the total use (relative use) from 1997-2005.**



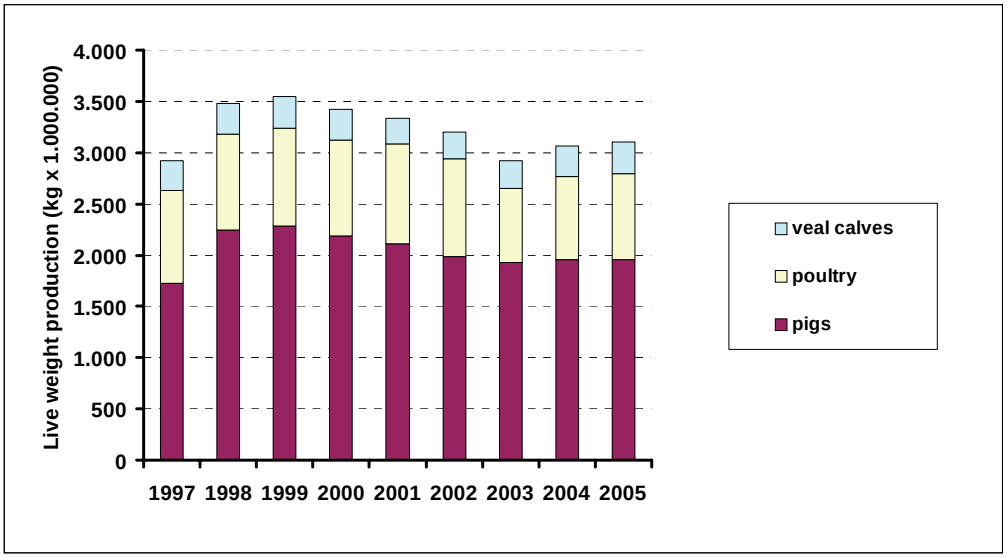

Source: FIDIN

**Figure 2. Developments in livestock (x 1000) in the Netherlands 1997 - 2005.**




**Source: Agricultural census, Statistics Netherlands (CBS).**

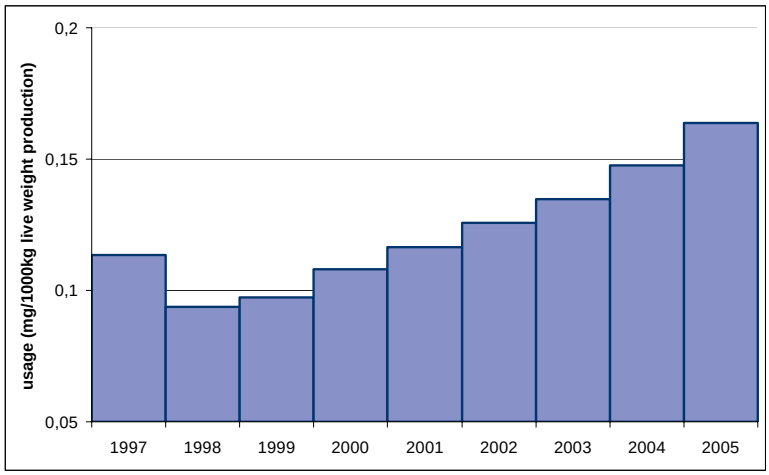
**Figure 3. Live weight production in the Netherlands 1997 - 2005.**




Factors influencing live weight production:
- February 1997: outbreak of swine fever
- February 2001: outbreak of feet and mouth disease
- February 2003: outbreak of avian influenza

Source: Product Boards for Livestock, Meat and Eggs (PVE)

**Figure 4. Total antibiotic usage (mg) per kg live weight production (pigs, poultry and veal calves) 1997-2005**



## Usage of antibiotics at dairy, pig and broiler farms (continuous monitoring)

The above-mentioned sales data from the pharmaceutical industry offer a general overview on antibiotic usage in the Netherlands. However, to obtain more detailed information, a continuous program to monitor antibiotic usage on farm level with data from the Agricultural Economics Research Institute (LEI) started in 2004. LEI is an institute in the Netherlands for social and economic research on agriculture, horticulture, fisheries, forestry and rural areas. LEI has developed the 'Farm Accountancy Data Network'. Various data from a random sample of agricultural and horticultural holdings are stored in this network. Based on this network economic data concerning veterinary medicines, originating from farm accountancies, were obtained. LEI has also detailed information regarding the exposed population, in the Farm Accountancy Data Network of LEI the average number of animals present at a farm during a certain year is being determined accurately. This data-combination was analysed in cooperation with the Pharmacy of the Faculty of Veterinary Medicine.

**Table 3. Characteristics of farms and animals included in 2005**

| Type of facility | Number of farms in sample | Type of Animal | Number of animals in sample | Percentage in sample (total number of animals in the Netherlands, LEI/CBS) |
|---|---|---|---|---|
| Dairy | 33 | Milking cows | 2.719 | 0,2 % (1.433.000) |
| Pigs | | Sows | 17.919 | 1,9 % (947.000) |
| | | Fattening pigs (> 20 kg) | 57.409 | 1,0 % (5.504.000) |
| Breeding | 26 | | | |
| Fattening | 15 | | | |
| Closed | 18 | | | |
| Broiler | 30 | Broilers | 2.134.153 | 4,8 % (44.496.000) |

The data in 2005 were based on 122 farms (Table 3): 33 dairy farms, 59 pig farms and 30 broiler farms. To be able to compare 2005 with 2004, the farms included in the sample were preferentially the same in 2004 as in 2005. Numbers of individual farms both included in 2004 and 2005 were 31 dairy farms, 44 farms with sows, 33 farms with fattening pigs and 15 farms with broilers.

In Table 5 and Figure 5 the number of doses per animal year is presented for intramammary treatment of dairy cattle (for an explanation of the unit of measurement; see Table 4) and the antibiotics used for systemic and intra-uterine treatment in dairy cattle and their calves are presented in Table 5 and Figure 6. The calculations are based on the average weight of the milking cows present at the farm; however antibiotics can also be administered to calves present at the farm. Milking cows are cows that have calved at least one time and are held for milk production or breeding purposes. The amount of intramammaries administered to milking cows was 3.35 doses per animal in 2005 compared to 3.74 doses in 2004. An explanation for this difference is not available. By parenteral, oral and intra-uterine administration 2.39 dd/ay were administered in 2005, this is the same amount as in 2004. The use of fluoroquinolones and macrolides was limited. The use of the third generation cephalosprines increased in 2005, ceftiofur is now among the most commonly used parenteral drugs in milking cows. This is related to the zero withdrawal time for milk of ceftiofur. The hypothesis that farms with high productive cows do have a higher use of antibiotics could not be confirmed; no relationship was found between average milk production per cow and antibiotic usage.

**Table 4. Antibiotics for systemic use: units of measurement for exposure (numerator) and population at risk (denominator)**

**Numerator**
Exposure data of veterinary drugs are often expressed in kilogram of active substance. In order not to underestimate the use of high potency drugs, the number of daily dosages (dd's) is preferably used as a unit of measurement. In order to calculate the number of dd's administered, the quantity of a veterinary medicinal product is divided by the approved dose for that medicine.
For example: 1 liter of Baytril ® 10% (100 mg/ml) is used in broilers; the approved dose is 10 mg/kg bodyweight per day. Thus 1 liter of the Baytril® solution represents 10.000 dosages to treat 1 kg of poultry during one day. Assuming that the average broiler weight is 1 kg, 1 liter of Baytril® solution can be used to treat 10.000 broilers during one day. 1 liter of Baytril® represents 10.000 dd's.

**Denominator**
To come to meaningful conclusions, the exposure to antibiotics must be related to the population at risk and the period of time over which consumption is measured. Estimations of livestock usually are a snapshot in time, reporting the number of animals that were present on a particular day. Assuming that the number of animals at risk is constant throughout the year, it could be calculated (depending on the number of animal housings) how many animals were at risk of being exposed to antibiotics during a certain period of time (in this case during one year).
For example: one pig is present and the antibiotic exposure was measured during one year. It is assumed that, although this pig was slaughtered within 6 months, there was one pig present throughout the entire year and that therefore the potentially exposed population (the population at risk) was one pig year (or 365 pig days). To report the population at risk, the words Animal Years (ay) or Animal Days (ad) are used.

As demonstrated in Table 6 and figure 7, the antibiotic usage on pig farms was substantially higher compared to dairy cattle (see also Figure 8). Antibiotic usage is concentrated in breeding facilities rather than in fattening facilities. Furthermore, in the breeding facilities the number of daily dosages was calculated over the total average weight of sows and piglets (and other pigs) present at a farm. We suppose however that antibiotics were administered more intensively to piglets than to sows. The total average weight of the piglets present at a farm amounts to some 15 - 20% of the weight of the sows present. Taking this into consideration, the real exposure of piglets to antibiotics will have beeen higher than calculated here. In particular trimethoprim/sulphonamide combinations and penicillins were used more intensively in breeding facilities. This may be related to usage in weaning piglets. Overall, tetracyclines were used often whereas the use of quinolones or fluoroquinolones was limited. Both in fattening pigs and in sows with piglets, antibiotic usage in 2005 was higher as compared to 2004. A significant positive relationship was found between the average number of piglets per sow and antibiotic usage at farms ($p = 0,014$) and also between the size of the farm (number of workers) and antibiotic usage ($p = 0,036$).

Antibiotic usage in broiler farms is presented in Table 8 and Figure 8. Broilers in this sample used 19.1 daily dosages per animal year in 2004 and 19.8 in 2005. This equals to 0.04 dosages per day. During their approximately 40-day life the average broiler in this sample was medicated with antibiotics for therapeutic purposes for almost 2 days. Tetracyclines as well as quinolones (flumequine only) and fluoroquinolones and trimethoprim/sulphonamide combinations are used relatively frequent. In Figure 9 the dd/ay are presented by animal species. Obviously to young piglets most often antibiotics were administered, with broilers at second and slaughter pigs at third place. Although in this study an increase in usage is recorded, this increase does not fully explain the increase in sales data provided by FIDIN. The difference in usage in daily dosages versus the usage in grams is of course an important factor here, but maybe also the lack of data about the use of antibiotics in veal calves may explain the observed difference.

**Table 5. Number of daily dosages per animal year (dd/ay) administered in dairy cattle (intramammary use), continuous monitoring programme (only farms sampled in 2004 and in 2005 are included)**

| Therapeutic Group | Intramammary use | dd/ay 2004 | dd/ay 2005 |
|---|---|---|---|
| Cephalosporins | Cefoperazone | 0,14 | 0,11 |
| | Cefquinome | 0,25 | 0,18 |
| Lincosamides | Pirlimycine | 0,02 | 0,00 |
| Penicillines | Cloxacillin | 0,98 | 0,97 |
| Combinations | Dihydrostreptomycin-benzylpenicillin-nafcillin | 0,45 | 0,38 |
| | Neomycin-benzylpenicillin | 0,42 | 0,50 |
| | Amoxicillin-clavulanic acid | 0,84 | 0,71 |
| | Ampicillin-cloxacillin | 0,09 | 0,00 |
| | Lincomycin-neomycin | 0,32 | 0,20 |
| | **Total milking cows** | **3,74** | **3,35** |

**Figure 5. Number of daily dosages per animal year (dd/ay) administered in dairy cattle (intramammary use) sampled in 2004 and in 2005**

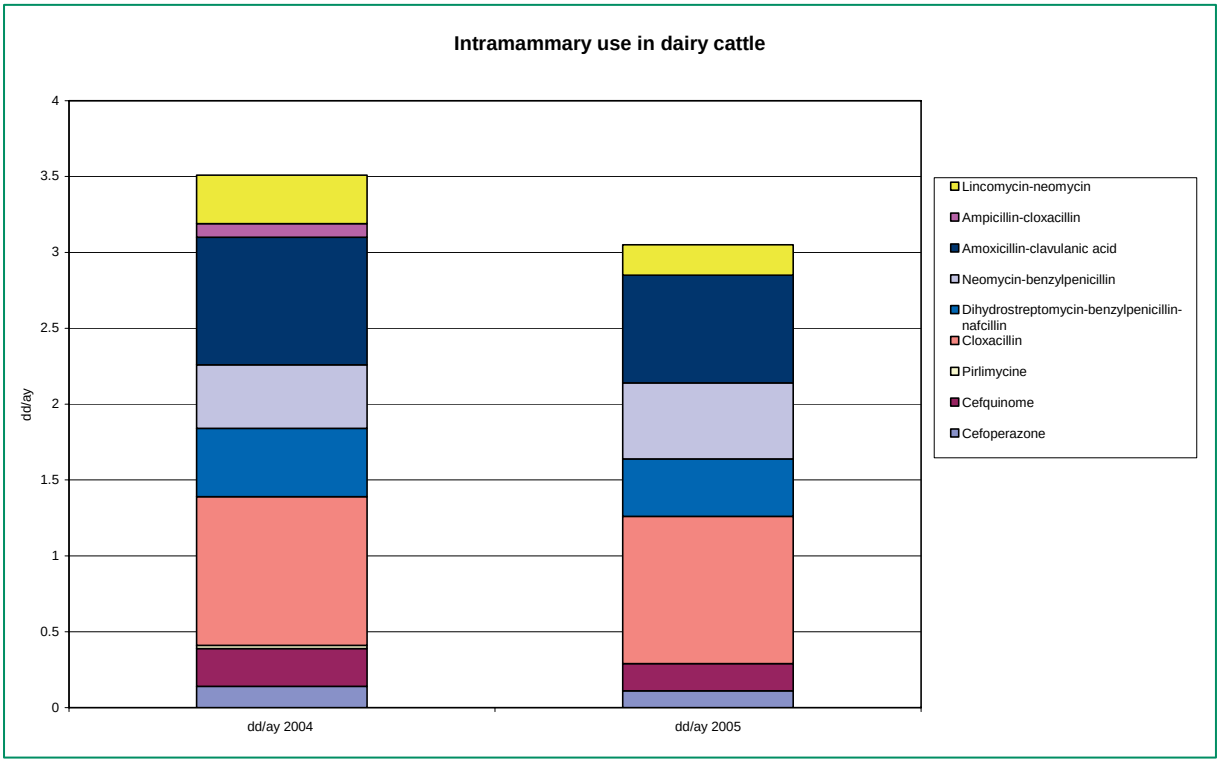


**Table 6. Number of daily dosages per animal year (dd/ay) administered in dairy cattle and their calves (intra-uterine, oral and parenteral administration), continuous monitoring programme (only farms sampled in 2004 and in 2005 are included)**

| Therapeutic group | **Active substance (administration)** | **dd/ay 2004** | **dd/ay 2005** |
|---|---|---|---|
| Cephalosporins | Cefquinome | 0,05 | 0,04 |
| | Ceftiofur | 0,19 | 0,31 |
| | Cefapirine | 0,03 | 0,04 |
| | | **0,27** | **0,39** |
| Penicillines | Benzylpenicillin | 0,42 | 0,27 |
| | Ampicillin | 0,13 | 0,17 |
| | | **0,55** | **0,44** |
| Macrolides and lincosamides | Erythromycin | 0,03 | 0,00 |
| | Tylosin | 0,01 | 0,10 |
| | Tilmycosine | 0,00 | 0,01 |
| | | **0,04** | **0,11** |
| Fluoroquinolones | Danofloxacin | 0,01 | 0,00 |
| | Enrofloxacin | 0,03 | 0,03 |
| | | **0,04** | **0,03** |
| Sulphonamides and trimethoprim | Trimethoprim-sulfadiazine | 0,03 | 0,05 |
| | Trimethoprim-sulfadoxine | 0,11 | 0,06 |
| | Trimethorpim -chloorpyridiazine | - | 0.01 |
| | | **0,14** | **0,12** |
| Tetracyclines | Doxycycline | 0,06 | 0,05 |
| | Chlortetracycline | 0,00 | 0,07 |
| | Oxytetracycline | 0,90 | 0,78 |
| | Tetracycline | 0,00 | 0,01 |
| | | **0,96** | **0,91** |
| Others | Florfenicol | 0,01 | 0,01 |
| | Lincomycin | 0,05 | 0,01 |
| | Neomycine | 0,01 | 0,01 |
| | Colistin | 0,05 | 0,09 |
| | | **0,12** | **0,12** |
| Combinations | Amoxicillin-colistin | 0,01 | 0,00 |
| | Dihydrostreptomycin-benzylpenicillin | 0,08 | 0,10 |
| | Neomycin-benzylpenicillin | 0,25 | 0,19 |
| | | **0,34** | **0,29** |
| | **Total** | **2,46** | **2,39** |

**Figure 6. Number of dd/ay used in dairy cattle and calves in 2004 and 2005 (i.u., p.o., i.m., s.c.)**

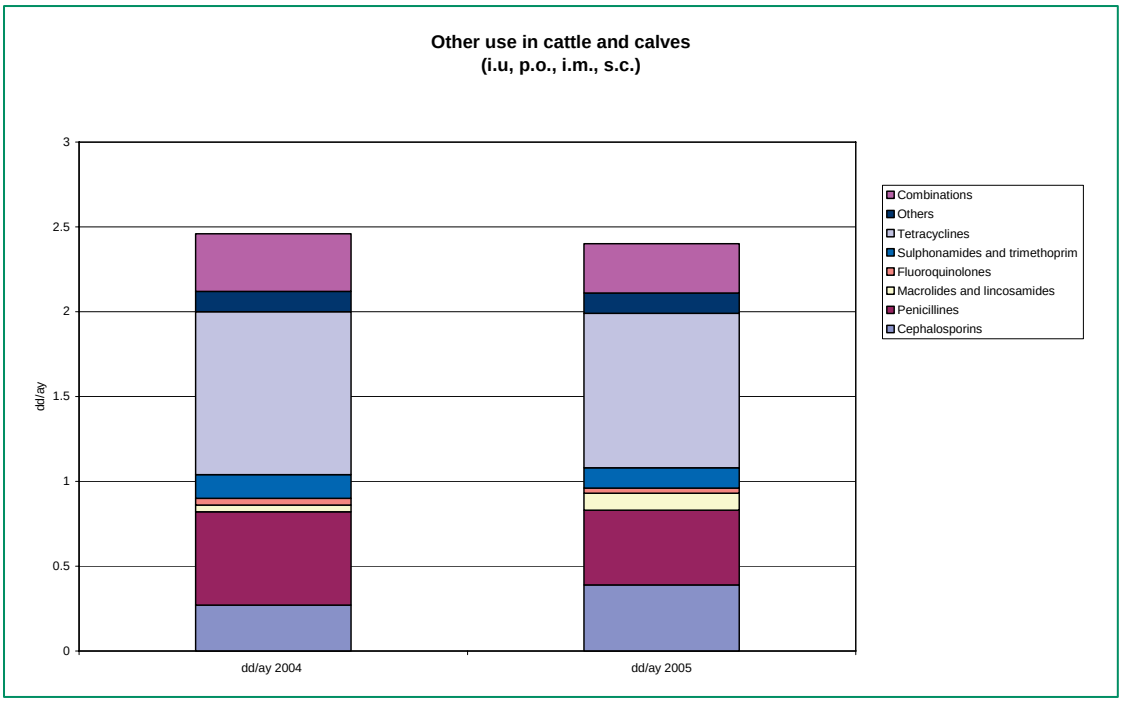



**Figure 7. Number of dd/ay used in pigs in 2004 and 2005.**

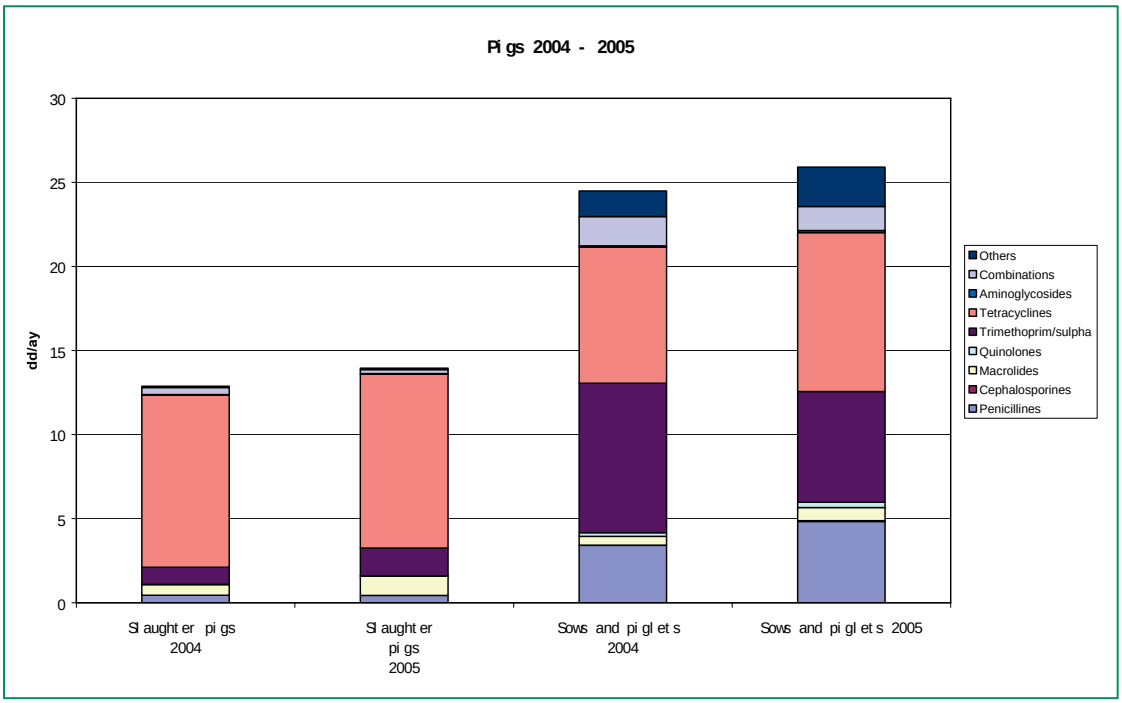


**Table 7. Average number of daily dosages per animal year (dd/ay) administered as group medication or individual (ind.) medication in fattening pigs and in sows inclusive piglets in 2006, continuous monitoring programme (only farms sampled in 2004 and in 2005 are included)**

| Therapeutic group | **Active substance** | Fattening pigs | | sows and piglets | |
|---|---|---|---|---|---|
| | | 2004 | 2005 | 2004 | 2005 |
| Penicillines | Benzylpenicillin | 0,29 | 0,30 | 0,93 | 0,77 |
| | Ampicillin | 0,12 | 0,12 | 0,85 | 0,80 |
| | Amoxicillin | 0,04 | 0,01 | 1,63 | 3,27 |
| | **Total** | **0,45** | **0,43** | **3,41** | **4,84** |
| Cephalosporines | Cefquinome | 0 | 0 | 0,01 | 0,02 |
| | Ceftiofur | 0 | 0 | 0,01 | 0,02 |
| | **Total** | **0** | **0** | **0,02** | **0,04** |
| Macrolides | Tilmicosin | 0,09 | 0,03 | 0,27 | 0,56 |
| | Tylosin | 0,55 | 1,12 | 0,25 | 0,17 |
| | Tulathromycine | - | 0,01 | - | 0,05 |
| | **Total** | **0,64** | **1,16** | **0,52** | **0,78** |
| Quinolones | Flumequine | 0 | 0 | 0,19 | 0,28 |
| | Enrofloxacin | 0,01 | 0,01 | 0,02 | 0,04 |
| | **Total** | **0,01** | **0,01** | **0,21** | **0,32** |
| Sulphonamides | Tmp-sulfadiazine | 0,63 | 1,09 | 4,77 | 2,13 |
| and trimethoprim | Tmp-sulfadoxine | 0 | 0 | 0,16 | 0,07 |
| | Tmp-sulfamethoxazole | 0,38 | 0,56 | 3,97 | 4,38 |
| | **Total** | **1,01** | **1,65** | **8,90** | **6,58** |
| Tetracyclines | Doxycycline | 5,33 | 5,00 | 2,32 | 3,72 |
| | Oxytetracycline | 4,92 | 5,34 | 5,77 | 5,73 |
| | **Total** | **10,25** | **10,34** | **8,09** | **9,45** |
| Aminoglycosides | Gentamicin | 0,02 | 0,03 | 0,08 | 0,11 |
| | Neomycin | 0 | 0 | 0 | 0,02 |
| | **Total** | **0,02** | **0,03** | **0,08** | **0,13** |
| Combinations | Lincomycin-spectinomycin | 0,02 | 0,01 | 0,52 | 0,58 |
| | Amoxicillin-colistin | 0,06 | 0,01 | 0,16 | 0,15 |
| | Neomycin-benzylpenicillin | 0,01 | 0,02 | 0,07 | 0,07 |
| | Dihydrostreptomycin-nezylpen-nafcilline | 0 | 0,21 | 0 | 0,11 |
| | Dihydrostreptomycin-benzylpenicillin | 0,33 | 0,20 | 0,99 | 0,62 |
| | **Total** | **0,42** | **0,24** | **1,74** | **1,42** |
| Others | Tiamulin | 0 | 0 | 0,02 | 0,00 |
| | Colistin | 0,05 | 0,08 | 1,48 | 2,33 |
| | Florfenicol | 0,02 | 0,01 | 0,02 | 0,01 |
| | **Total** | **0,07** | **0,09** | **1,52** | **2,34** |
| **Total** | | **12,87** | **14,39** | **24,48** | **26,01** |

**Table 8. Average number of daily dosages per animal year (dd/ay) administered in broiler farms, continuous monitoring programme (only farms sampled in 2004 and in 2005 are included)**

| Therapeutic group | ***Active substance*** | dd/ay 2004 | dd/ay 2005 |
|---|---|---|---|
| Penicillines | Ampicillin | 0,41 | 0,09 |
| | Amoxicillin | 2,42 | 3,14 |
| | **Total** | **2,83** | **3,23** |
| Macrolides and lincosamides | Tylosin | 0,77 | 1,08 |
| Quinolones | Enrofloxacin | 0,33 | 0,21 |
| | Flumequine | 3,92 | 5,26 |
| | **Total** | **4,25** | **5,47** |
| Sulphonamides and trimethoprim | Trimethoprim-sulfachloorpyridazine | 1,56 | 1,72 |
| | Trimethoprim-sulfamethoxazole | 1,89 | 1,46 |
| | Sulfadimidine | 0,07 | 0,03 |
| | **Total** | **3,55** | **3,21** |
| Tetracyclines | Doxycycline | 5,38 | 2,02 |
| | Oxytetracycline | 1,82 | 2,07 |
| | **Total** | **7,20** | **4,09** |
| Aminoglycosides | Neomycin | 0,46 | 2,55 |
| Combinations | Lincomycin-spectinomycin | 0,04 | 0,17 |
| **Total** | | **19,10** | **19,81** |

**Figure 8. Number of dd/ay used in broilers in 2004 and 2005.**

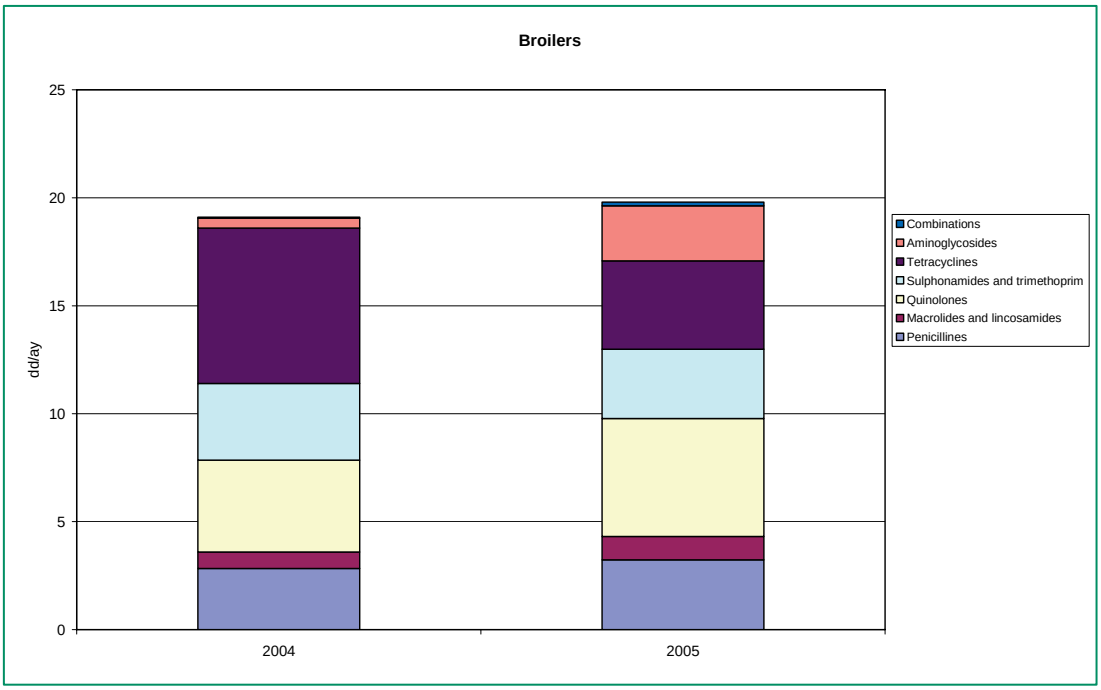
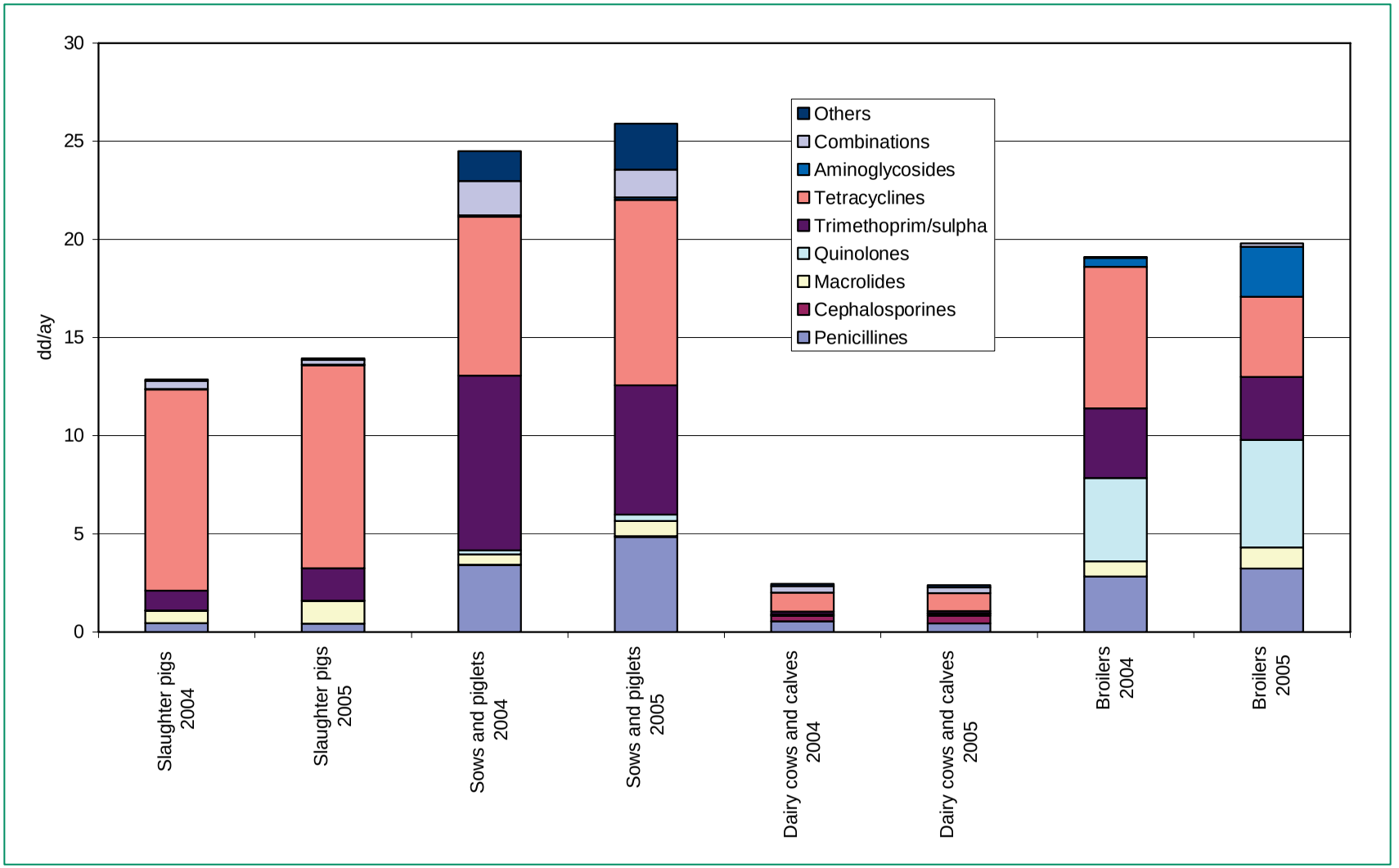


**Figure 9. Numbers of dd/ay used in cattle, pigs and broilers in 2004 and 2005 in The Netherlands.**



# II Resistance data

In this chapter susceptibility test results are presented as determined in 2005 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*, the bovine mastitis pathogens *Staphylococcus aureus*, coagulase negative staphylococci, *Streptococcus uberis*, *S. dysgalactiae*, *E. coli* and coliform bacteria, and the bovine respiratory disease pathogens *Pasteurella multocida* and *Mannheimia haemolytica.*

## *Food-borne pathogens*

### *Salmonella* spp.

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 2005 for the first time since the 80's, *S.* Typhimurium was again the most prevalent serovar in humans. This is mainly the result of a large outbreak with 165 extra cases of salmonellosis caused by DT104 in autumn 2005 related to imported beef. *S.* Enteritidis was still the second most prevalent serovar in humans. Pigs and cattle were the most important animal sources of *S.* Typhimurium. In layers (eggs) an increase in incidence of *S.* Enteritidis was observed. In broilers *S.* Java was isolated most frequently. In broilers *S.* Enteritidis and *S.* Typhimurium constitute only a small fraction of all salmonella's.

Resistance levels in *S.* Enteritidis showed a dramatic change in PT4 from Dutch layers. For the first year a high percentage of quinolone resistance was observed in this sero/phage type from layers. This is most probably related to import of resistant organisms and not by selection through usage of (fluoro)quinolones in these animals. High level resistance to ciprofloxacin was only incidentally detected in *S.* Kentucky strains isolated from human patients (also detected in 2002, 2003 and 2004). These strains were related to travel to North African countries and not to Dutch food-animals. Quinolone resistance in Enteritidis from human patients was predominantly related to PT1 and to a lesser extent to PT4. This indicates that quinolone resistant strains of *S.* Enteritidis isolated from humans predominantly originate from imported animal products or from travel related infections.

Striking is the increase in resistance to modern third-generation cephalosporins in salmonella's from poultry. This is most likely caused by transfer of resistance plasmids from commensal *E. coli* in these animals.

The decrease of the prevalence of *S.* Java in broilers is an artefact due to selective submission of isolates for typing by the poultry industry; at retail the proportions of poultry meat products contaminated with *S.* Java remained at the same high levels before.

For the purpose of antimicrobial resistance surveillance in *Salmonella* spp., it is essential to include information on the relative importance of the different serovars in humans, food-animals and animal feed (table 9). In 2005, *S.* Typhimurium was the most prevalent serovar isolated from humans in The Netherlands. The reason is that in 2005 a large outbreak occurred with *S.* Typhimurium DT104 with a cluster of 165 extra cases of DT104 strains isolated from humans infected by consumption of contaminated imported beef (Marten Kivi et. al., 2005: Eurosurveillance, Dec. 2005). The strains were identical to an outbreak cluster of DT104 in Denmark two months earlier, involving the same batch of contaminated beef.(Ethelberg, Eurosurveillance, Sept. 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 *Salmonella* spp. between broilers and layers. In isolates from broilers *S.* Paratyphi B var. Java (*S.* Java) and *S.* Infantis predominated and in layers *S.* Enteritidis. Travel contributed from 0% to almost 50% 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 with a high level of resistance to ciprofloxacin.

**Table 9. Most prevalent *Salmonella* sero-, and phagetypes isolated in 2005 (2004 between brackets) from humans, pigs, poultry, broilers and layers[2] and the % travel related infections in 2004 – 2005.**

| | | Human | Pigs | Cattle | Poultry | Broilers | Layers |
|---|---|---|---|---|---|---|---|
| **Total number sent to RIVM** | | **1621** | **269** | **97** | **863** | **203** | **506** |
| **Sero/phagetype** | **Travel%** | **% of the total sent to RIVM (2004 data between brackets)** | | | | | |
| Typhimurium | 3% | 40,2 (28,5) | 64,7 (47,9) | 14,4 | 8,2 (3,5) | 9,9 (3,9) | 7,3 (2,8) |
| DT104 | 2% | 23,8 | 20,4 | 4,1 | 3,4 | 3,9 | 2,8 |
| Ft507 | 2% | 7,2 (6,4) | 14,9 (12,4) | 6,2 (3,2) | 1,7 (0,2) | 4,9 (0,3) | 0,8 |
| Ft510 | 8% | 1,4 | 0,4 | 1,0 | --- | --- | --- |
| Ft290 | 13% | 0,4 | --- | --- | --- | --- | --- |
| Ft80 | 7% | 0,2 | 1,1 | --- | --- | --- | --- |
| Ft60 | 0% | 0,1 | 1,9 | --- | --- | --- | --- |
| Enteritidis | 11% | 33,7 (47,2) | --- | 1,0 | 26,1 (9,5) | 9,4 (5,9) | 35,2 (22) |
| Pt 4 | 8% | 10,4 (13,8) | --- | --- | 7,8 (3,9) | 1,0 | 11,3 (8,5) |
| Pt 21 | 10% | 5,8 | --- | --- | 3,0 | 4,9 | 3,2 |
| Pt 6 | 11% | 3,6 | --- | --- | 1,9 | --- | 2,6 |
| Pt 1 | 18% | 3,2 | --- | --- | 2,2 | 1,5 | 2,2 |
| Pt 8 | 7% | 2,6 (6,2) | --- | 1,0 | 3,2 | 1,0 | 3,8 |
| Pt 14b | 14% | 1,7 | --- | --- | 0,3 | 0,5 | 0,4 |
| Pt 6a | 20% | 0,9 (1,8) | --- | --- | 0,9 | --- | 1,6 |
| Pt 7 | 13% | 0,2 | --- | --- | 2,4 | --- | 3,4 |
| Dublin | 0% | 0,3 | --- | 70,1 (44,9) | 0,2 | --- | --- |
| Derby | 10% | 1,0 | 17,8 | --- | 0,6 | 0,5 | 0,8 |
| Infantis | 13% | 1,4 | 1,9 | 1,0 | 10,3 | 24,6 (7,6) | 4,9 |
| Paratyphi B var Java | 0% | 0,1 | 0,4 | --- | 10,8 (23,6) | 22,7 (34,6) | 1,8 |
| Senftenberg | 29% | 0,5 | --- | --- | 7,5 | 2,5 | 11,1 (25,5) |
| Mbandaka | 14% | 0,1 | --- | 3,1 | 3,7 | 2,5 | 4,7 |
| SI 1,4,5,12:i:2ef nat | 4% | 1,7 | 0,7 | 4,1 | 0,3 | 1,0 | --- |
| Virchow | 31% | 1,4 | --- | --- | 4,2 | 3,4 | 3,6 |
| Brandenburg | 3% | 1,6 | 3,0 | -2,7 | 0,3 | --- | 0,6 |
| Livingstone | 6% | 0,2 | 2,6 | --- | 1,9 | 1,5 | 2,4 |
| Rissen | 0% | 0,1 | 3,0 | --- | 0,3 | --- | 0,6 |
| Kentucky | 46% | 0,9 | --- | --- | 2,3 | 2,5 | 2,8 |
| Goldcoast | 2% | 0,2 | 0,4 | 2,1 | 0,2 | 0,5 | 0,2 |
| Agona | 21% | 0,1 | 0,4 | --- | 2,3 | 1,0 | 3,0 |
| Corvallis | 24% | 1,4 | --- | --- | 1,0 | 0,5 | 0,6 |
| Hadar | 22% | 1,0 | --- | --- | 1,2 | 1,5 | 0,8 |
| Bovismorbificans | 4% | 0,2 | 0,4 | 1,0 | 0,5 | 1,0 | 0,4 |
| Saintpaul | 29% | 1,3 | --- | --- | 0,8 | 2,0 | 0,2 |
| Braenderup | 0% | 0,3 | --- | --- | 1,4 | --- | 2,4 |
| Anatum | 29% | 0,4 | 0,4 | --- | 0,7 | 2,5 | 0,2 |
| Montevideo | 18% | 0,3 | --- | --- | 1,0 | --- | 1,6 |
| Tennessee | 0% | 0,1 | --- | --- | 1,2 | 0,5 | 1,8 |
| (Para)Typhi (A,B,C) | 30% | 1,3 | --- | --- | --- | --- | --- |
| Other serotypes | | 22,9 | 30,5 | 6,2 | 20,3 | 11,8 | 23,9 |

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.

The decreasing occurrence of *S.* Java in broilers is due to selective submission of isolates for typing by the poultry industry. However, *S.* Java is still by far the most frequently isolated serovar at retail from broiler products (Table 15) indicating that the contamination rate in broilers is at the same high level as before.

[2] Source: Report on trends and sources of zoonotic agents in the EU, 2005, The Netherlands

**Table 10. MIC distribution (in %) for all salmonella's (N = 2238) tested for antibiotic susceptibility in 2005.**

| | MIC (%) distribution (mg/L) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total 2005** | **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%** |
| Amoxicillin | | | | | | 23,6 | 59,1 | 1,3 | 0,04 | 0,1 | 0,04 | | | 15,8 | | | | | 16,0 |
| Cefotaxim | | | | 90,2 | 8,2 | 1,0 | 0,1 | | 0,1 | | | 0,4 | | | | | | | 0,7 |
| Ceftazidime | | | | 22,1 | 63,0 | 13,2 | 1,0 | 0,1 | 0,2 | | 0,1 | 0,3 | | | | | | | 0,7 |
| Imipenem | | | | 74,7 | 23,4 | 1,7 | 0,2 | | | | | | | | | | | | 0,0 |
| Gentamicin | | | | | 33,2 | 57,4 | 7,9 | 0,5 | | 0,2 | 0,3 | 0,1 | 0,4 | | | | | | 0,9 |
| Neomycin | | | | | | | 92,0 | 6,5 | 0,1 | 0,1 | | 0,2 | 0,4 | 0,4 | 0,2 | | | | 1,3 |
| Tetracycline | | | | | | 0,3 | 17,8 | 59,5 | 4,0 | 0,8 | 0,1 | 5,4 | 3,3 | 8,9 | | | | | 17,7 |
| Sulphamethoxazole | | | | | | | | | | 39,3 | 42,8 | 0,8 | | | | | 0,04 | 17,1 | 17,1 |
| Trimethoprim | | | | | | 89,2 | 3,5 | 0,2 | | | | | | 7,1 | | | | | 7,1 |
| Ciprofloxacin | | | 89,9 | 2,5 | 4,2 | 2,7 | 0,6 | 0,1 | | | 0,1 | | | | | | | | 10,1 |
| Nalidixic acid | | | | | | | | 8,4 | 75,8 | 5,8 | 1,4 | | | 0,9 | 7,7 | | | | 8,7 |
| Chloramphenicol | | | | | | | | | 2,4 | 73,8 | 15,4 | 0,2 | | 0,5 | 7,6 | | | | 8,4 |
| Florfenicol | | | | | | | | 0,4 | 38,2 | 52,3 | 2,5 | 2,1 | 2,9 | 0,9 | 0,8 | | | | 6,6 |

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. The vertical bars indicate the breakpoints.

Table 10 presents MIC-distributions and resistance percentages of all salmonella's tested for susceptibility in 2005. Highest levels of resistance were observed for sulphamethoxazole, tetracycline and amoxicillin and to a lesser extend ciprofloxacin, nalidixic acid, trimethoprim and chloramphenicol.
Fifteen cefotaxime resistant, ESBL suspected strains were found, which was more than in 2004 (n = 7). The isolates belonged to the following serovars: 4 *S.* Java and 1 Kentucky from poultry, 1 *S.* Braenderup from animal feed, and 1 *S.* Richmond, 1 *S.* Concord, 1 *S.* Heidelberg, 1 *S.* Weltevreden, *S.* Typhimurium FT 656, 1 *S.* Enteritidis PT 34, 1 *S.* Hadar and 1 *S.* Infantis all isolated from human patients. Three of these isolates (2 Java, Hadar) also showed reduced susceptibility to ciprofloxacin. One isolate (Enteritidis PT34) also showed high level ciprofloxacin resistance. It is the fourth consecutive year that ESBL-positive *S.* Java strains were detected in broilers. This is likely to be related to transfer of plasmid mediated ESBLs from *E. coli* in the gastro-intestinal tract in broilers.

Twenty-one gentamicin resistant strains, and twenty-nine neomycin resistant strains were found, the majority isolated from human patients. Eight of the neomycin resistant strains were *S.* Typhimurium FT 507.

Using the epidemiological cut off value of 0.06 mg/L, 227 strains were detected that demonstrated a non-wild type phenotype for ciprofloxacin. However only three isolates demonstrated high-level ciprofloxacin resistance: 2 *S.* Kentucky and 1 *S.* Enteritidis PT34 also ESBL positive. 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 *aac*C-A5 and *aad*A7 encoding for aminoglycoside resistance.

In 2005 17 isolates of which 14 from human patients demonstrated an atypical quinolone resistance phenotype, these strains showed reduced susceptibility to ciprofloxacin (MIC 0.5 µg/ml) but were susceptible to nalidixic acid (MIC 16 µg/ml). Eight of the seventeen isolates were *S.* Corvallis. The genetic basis of this phenotype is yet unknown.

Nine fully susceptible *S.* Newport strains were found, one isolated from an unknown source, eight from human patients.

**Table 11. Resistance (%) of the twelve most prevalent *Salmonella* serovars isolated in The Netherlands in 2005.**

| | Typhimurium (603) | Enteritidis (568) | Senftenberg (129) | Dublin (71) | Infantis (67) | Mbandaka (61) | Agona (47) | Rissen (43) | Kentucky (42) | Livingstone (41) | Lexington (39) | Virchow (36) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amoxicillin | 56,0 | 2,1 | 2,3 | 0 | 6,0 | 4,9 | 2,1 | 2,3 | 11,9 | 4,9 | 0 | 2,8 |
| Cefotaxim | 0,2 | 0,2 | 0 | 0 | 1,5 | 0 | 0 | 0 | 2,4 | 0 | 0 | 0 |
| Ceftazidime | 0 | 0,3 | 0 | 0 | 2,0 | 0 | 0 | 0 | 3,3 | 0 | 0 | 0 |
| Imipenem | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 0,6 | 0,4 | 0 | 0 | 1,5 | 1,6 | 0 | 0 | 4,8 | 0 | 0 | 0 |
| Neomycin | 2,3 | 0,2 | 0 | 1,5 | 1,5 | 0 | 0 | 2,3 | 2,4 | 0 | 0 | 2,8 |
| Tetracycline | 60,4 | 1,2 | 1,6 | 0,0 | 3,0 | 1,6 | 0 | 2,3 | 14,3 | 7,3 | 0 | 25,0 |
| Sulphamethoxazole | 56,8 | 1,6 | 3,1 | 4,6 | 4,5 | 3,3 | 2,1 | 2,3 | 7,1 | 14,6 | 0 | 22,2 |
| Trimethoprim | 16,8 | 1,1 | 1,6 | 0 | 4,5 | 3,3 | 2,1 | 2,3 | 2,4 | 9,8 | 0 | 19,4 |
| Ciprofloxacin | 3,6 | 17,4 | 1,6 | 3,1 | 6,0 | 1,6 | 0 | 0 | 9,5 | 0 | 0 | 77,8 |
| Nalidixic acid | 2,3 | 15,5 | 0,8 | 4,6 | 4,5 | 1,6 | 0 | 0 | 9,5 | 0 | 0 | 77,8 |
| Chloramphenicol | 32,4 | 0,4 | 1,6 | 4,6 | 3,0 | 1,6 | 0 | 2,3 | 0 | 2,4 | 0 | 2,8 |
| Florfenicol | 28,6 | 0,4 | 1,6 | 0 | 0 | 0 | 0 | 2,3 | 0 | 0 | 0 | 0 |

In table 11 resistance percentages are presented for the twelve most prevalent serovars isolated in The Netherlands in 2005. The highest resistance levels are observed in *S.* Typhimurium, *S.* Kentucky and *S.*Virchow.

## *S.* Enteritidis

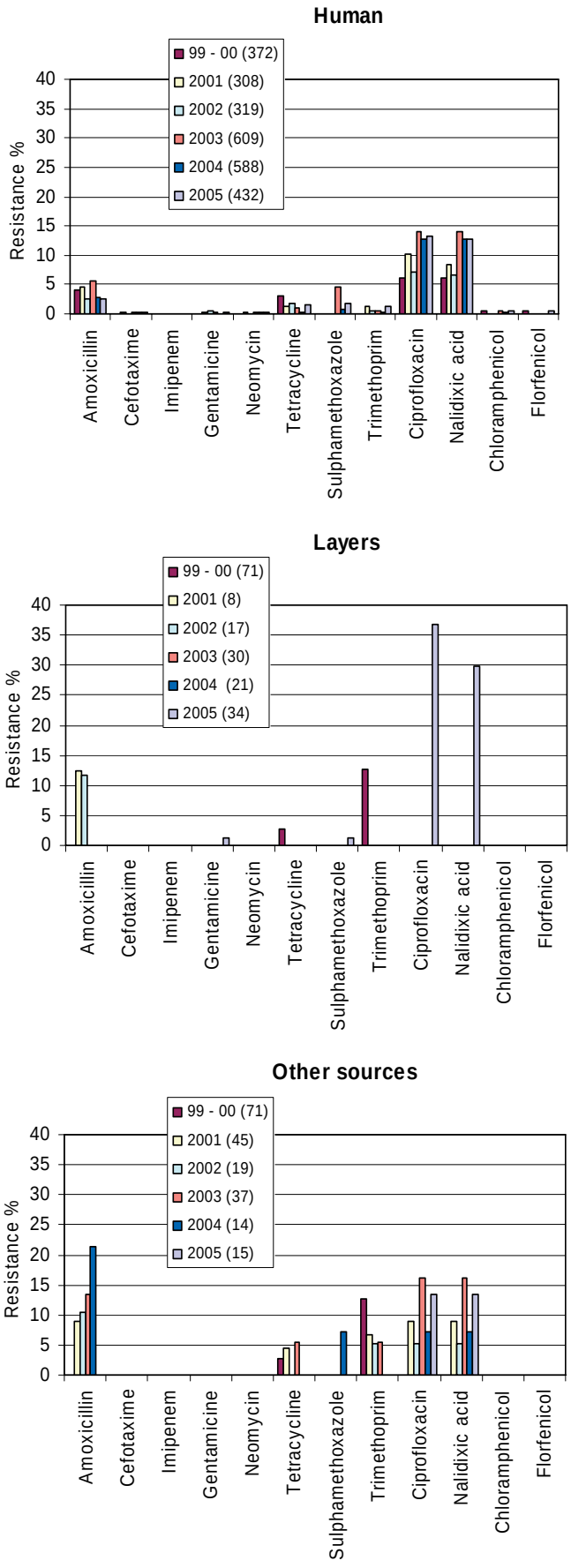
In table 12 resistance percentages for *S.* Enteritidis and it most prevalent phage types are presented. In The Netherlands, human infections caused by *S.* Enteritidis are predominantly related to the consumption of raw shell eggs. In Dutch broilers and broiler products the prevalence of *S.* Enteritidis is substantially lower (Tables 9 and 15). The difference in phagetype distribution and resistance profile of strains from human infections and Dutch poultry indicates that other sources of infection exist. In 2005 from human infections, 60 ciprofloxacin non wild type susceptible strains were isolated, predominantly Pt1 (52%) and to a lesser extend Pt4 (20%). 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, indicating that eggs from Dutch layers might not be the most important source for human infections with this serovar.
The sudden high level of resistance in *S.* Enteritidis Pt4 from layers is 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 is introduction of quinolone resistant *S.* Enteritidis Pt4 by importation of contaminated eggs or breeding animals.

**Table 12. Resistance (%) of *S.* Enteritidis and phagetypes 4, 21, 6, 1, 8, 14b and 6a isolated from different sources in 2005.**

| | S. Enteritidis | | | Most prevalent phage types | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Human (449) | Layers (87) | Other poultry (15) | pt4 (204) | pt21 (92) | pt6 (65) | pt1 (54) | pt8 (48) | pt14b (25) | pt6a (15) |
| Amoxicillin | 2,7 | 0 | 0 | 0 | 0 | 3,1 | 1,9 | 2,1 | 0 | 40,0 |
| Cefotaxime | 0,2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftazidime | 0,3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Imipenem | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 0,2 | 1,1 | 0 | 0 | 0 | 0 | 0 | 2,1 | 0 | 0 |
| Neomycin | 0,2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6,7 |
| Tetracycline | 1,6 | 0 | 0 | 0 | 0 | 3,1 | 3,7 | 0 | 0 | 6,7 |
| Sulphamethoxazole | 1,8 | 1,1 | 0 | 0,5 | 0 | 3,1 | 3,7 | 2,1 | 0 | 0 |
| Trimethoprim | 1,3 | 0,0 | 0 | 0 | 0 | 3,1 | 3,7 | 2,1 | 0 | 0 |
| Ciprofloxacin | 13,4 | 36,8 | 13,3 | 17,2 | 4,3 | 0 | 64,8 | 0 | 28,0 | 46,7 |
| Nalidixic acid | 12,7 | 29,9 | 13,3 | 13,2 | 4,3 | 0 | 61,1 | 0 | 28,0 | 40,0 |
| Chloramphenicol | 0,4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Florfenicol | 0,4 | 0 | 0 | 0 | 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).
It can be concluded that quinolone resistant strains of *S.* Enteritidis isolated from humans primarily originate from other sources than Dutch poultry, like imported eggs or from travel related infections.

**Figure 10. Trends in resistance (%) of *S.* Enteritidis isolated from humans, layers and other poultry sources from 1999 – 2005**



## *S.* Typhimurium

In 2005 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 510 (table 13). In this collection only one of the isolates of the DT104 clone from the large autumn outbreak was included.

Although in *S.* Typhimurium multi drug resistance is commonly present (Fig. 11), resistance to quinolones occurs less frequently and ESBL suspected strains are rare, in comparison to other serovars.

In 2005 11 non ciprofloxacin wild type *S.* Typhimurium DT104 isolates were found from various sources. None of these isolates was high-level ciprofloxacin resistant.

Resistance levels and multiple resistances are substantially higher in *S.* Typhimurium than in *S.* Enteritidis (Table 11, figures 10 and 12). Of the strains, 52% (humans), 60%(pigs), 62% (poultry) and 67% (cattle) were resistant to three or more antibiotic classes (Fig. 11).

Trends in resistance in *S.* Typhimurium show a tendency to increase in strains from all sources (Fig. 12). However, 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 complexity of the trend analysis.

**Table 13. Resistance percentages of S. Typhimurium and phage types DT104, Ft 507, Ft401 and FT510 isolated from different sources in 2005.**

| | S. Typhimurium | | | | Phage types | | | |
|---|---|---|---|---|---|---|---|---|
| | Human (304) | Cattle (12) | Pigs (85) | Poultry (34) | DT104 (155) | Ft 507 (144) | FT 401 (20) | FT 510 (20) |
| Amoxicillin | 55,9 | 75,0 | 60,0 | 64,7 | 83,9 | 50,7 | 75,0 | 40,0 |
| Cefotaxime | 0,3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceftazidime | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Imipenem | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gentamicin | 1,0 | 0 | 0 | 0 | 0 | 0,7 | 0 | 5,0 |
| Neomycin | 3,0 | 8,3 | 0 | 2,9 | 0 | 5,6 | 0 | 5,0 |
| Tetracycline | 60,5 | 58,3 | 70,6 | 64,7 | 87,1 | 53,5 | 85,0 | 50,0 |
| Sulphamethoxazole | 56,3 | 75,0 | 63,5 | 64,7 | 86,5 | 50,0 | 75,0 | 40,0 |
| Trimethoprim | 14,1 | 33,3 | 31,8 | 8,8 | 16,8 | 20,8 | 0 | 10,0 |
| Ciprofloxacin | 2,6 | 8,3 | 2,4 | 11,8 | 7,1 | 2,8 | 0 | 5,0 |
| Nalidixic acid | 1,6 | 8,3 | 0,0 | 8,8 | 5,2 | 1,4 | 0 | 5,0 |
| Chloramphenicol | 29,6 | 33,3 | 40,0 | 44,1 | 76,1 | 6,3 | 0 | 20,0 |
| Florfenicol | 25,0 | 25,0 | 37,6 | 41,2 | 75,5 | 0,7 | 0 | 5,0 |

**Figure 11. Percentages of S. Typhimurium strains fully susceptible, resistant to one to nine different antibiotic classes in humans, pigs, cattle and poultry in The Netherlands in 2005.**

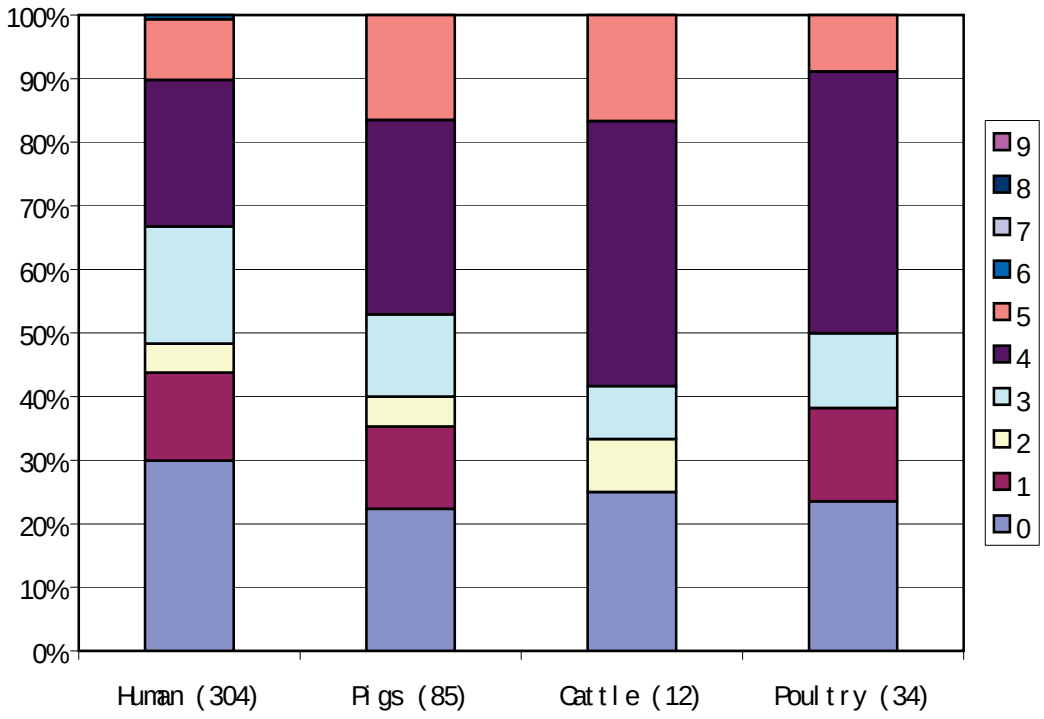
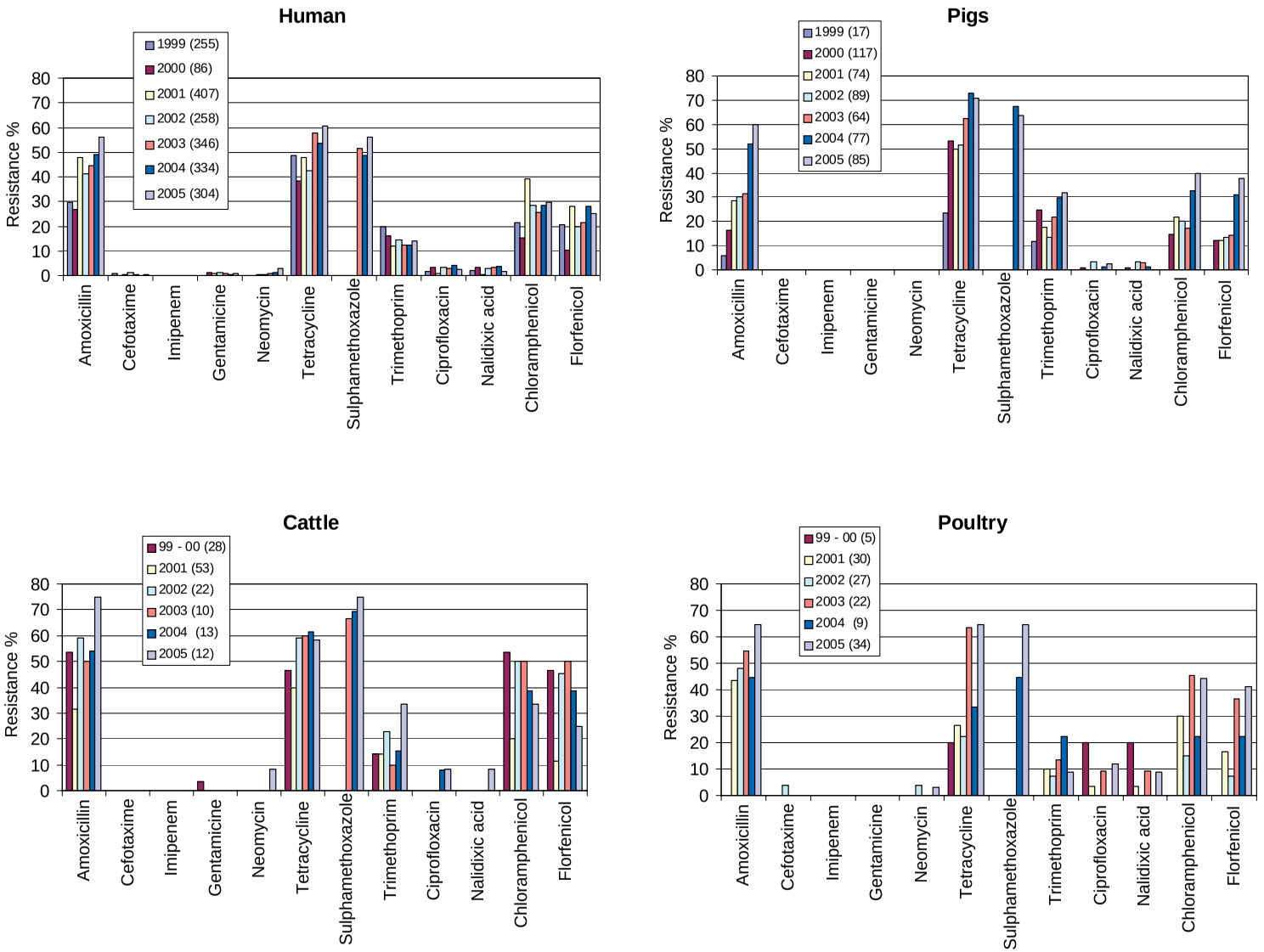


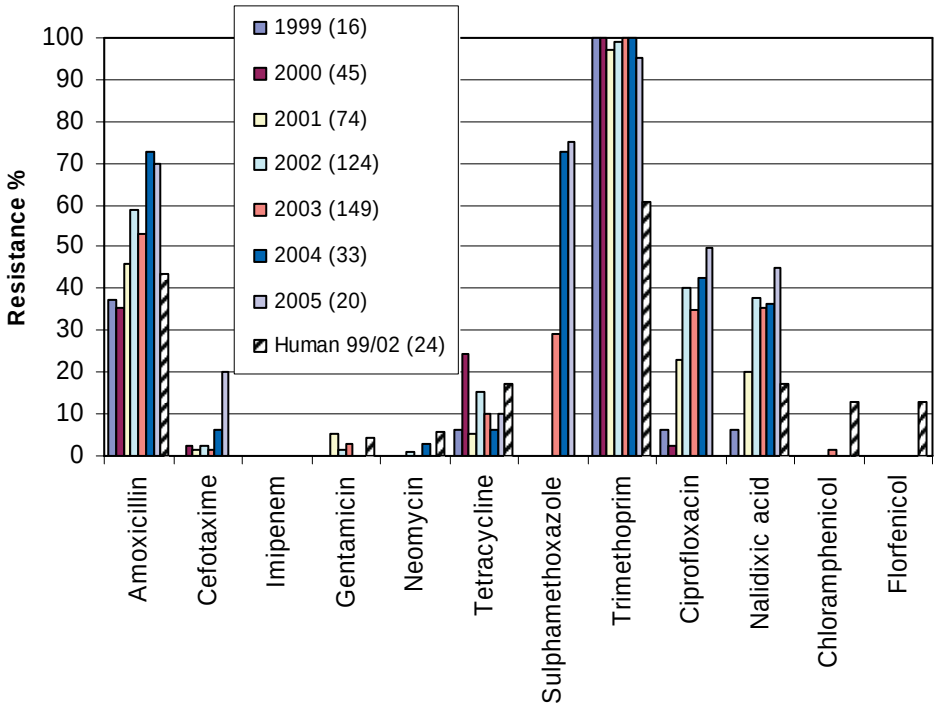
**Figure 12. Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals from 1999 - 2004**



## *S.* Paratyphi B var. Java

The prevalence data from the Dutch salmonella reference laboratory (RIVM) in the 2005 zoonoses report indicates a decrease in prevalence of *S.* Java in 2005 from 34.6% in 2004 to 22.2% in 2005. At retail however (statistical sampling, table 15) almost 50% and no clear decrease is observed. This indicates that the presence of this serovar in broilers is underreported due to selective submission of isolates for typing to the RIVM. In 2005 only once *S.* Java was isolated from a human infection. However, this isolate was fully susceptible to all antibiotics in the panel and therefore not related to the clone spreading in Dutch poultry.
From poultry 19 strains were isolated all harbouring the phenotype typical for the clone. Non wild type susceptibility to ciprofloxacin in *S.* Java isolated from poultry increased at least up to 2002 (figure 13). The small sample analysed in 2005 does not allow a firm conclusion of a further increase in the level of resistance nor is this supported by the findings in broiler products at retail (table 14, figure 14). No high-level ciprofloxacin resistant strains were found. Resistance to cefotaxime (ESBL-producers) shows a clear tendency to increase in 2004 and 2005, at retail as well. This is probably 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 poultry, therefore the use of other beta-lactam antibiotics or even other classes of antibiotics may co-select for beta-lactamases in multi drug resistant isolates.

**Figure 13. Trends in resistance (%) of *S.* Paratyphi B var. Java isolated from poultry from 1999 – 2005 and humans (Grey-white dashed bars indicate all humans isolates from 1999 – 2005 (N = 24))**



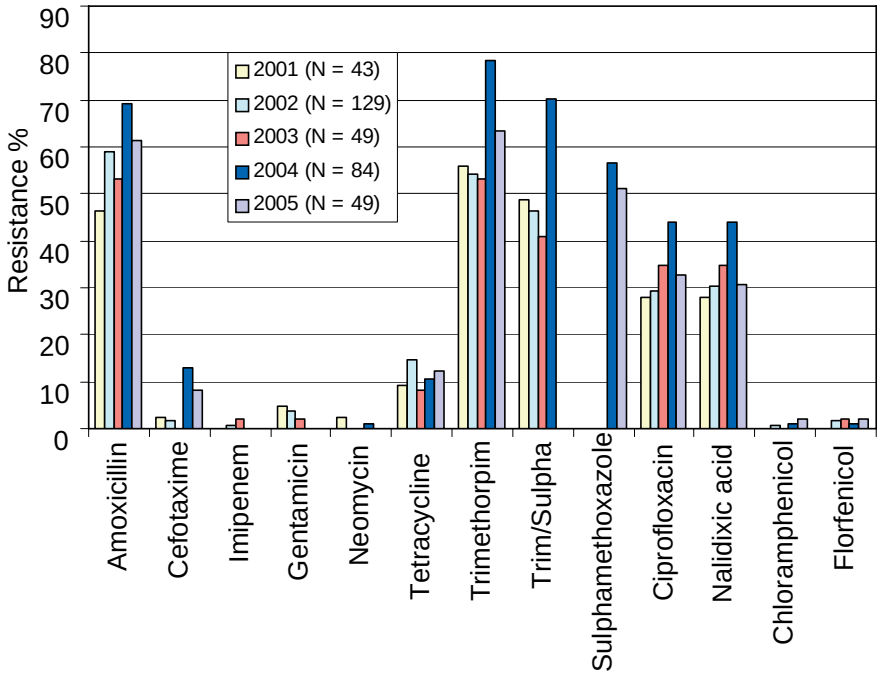
## *Salmonella* spp. in raw meat products of food-animals

**Table 14. Resistance (%) of *Salmonella* spp. isolated from raw meat from poultry, and other raw meat sources in 2005.**

| | Poultry S. Java N = 25 | Poulty other serovars N = 25 | Other* N = 13 |
|---|---|---|---|
| Amoxicillin | 80,0 | 40,0 | 33,3 |
| Cefotaxime | 12,0 | 4,0 | 0 |
| Imipenem | 0 | 0 | 0 |
| Gentamicin | 0 | 0 | 8,3 |
| Neomycin | 0 | 0 | 0 |
| Tetracycline | 8,0 | 16,0 | 25,0 |
| Sulphamethoxazole | 72,0 | 28,0 | 25,0 |
| Trimethoprim | 100 | 24,0 | 0 |
| Ciprofloxacin | 24,0 | 44,0 | 0 |
| Nalidixic acid | 24,0 | 36,0 | 0 |
| Chloramphenicol | 0 | 4,0 | 8,3 |
| Florfenicol | 0 | 4,0 | 8,3 |

* 5 pork, 3 beef, 4 hare, 1 wild duck

**Figure 14. Trends in resistance (%) of *Salmonella* spp. isolated from poultry products in the Netherlands from 2001 – 2005.**



In 2005 the majority of the salmonella's from raw meat products originated from poultry. *S.* Java is still by far the most prevalent serovar isolated. Because this serovar has a specific resistance profile, its results are presented separately. As expected, its resistance profile is similar to that of the strains isolated from broiler caecal content. Cefotaxime resistance is very commonly present. To a lesser extent cefotaxime resistance is also observed in other serovars from poultry meat sources. Horizontal transmission of beta-lactamases from commensal *E. coli* from broilers is the most likely explanation. Resistance trends are presented for poultry products because in beef and pork the numbers of isolates examined are too small to provide an accurate estimate (Fig. 14). The variable contribution of *S.* Java to the annual resistance percentages over all serotypes hampers the interpretation of the observed trend in the resistance.

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

| | **1997** | **1998** | **1999** | **2000** | **2001** | **2002** | **2003** | **2004** | **2005** |
|---|---|---|---|---|---|---|---|---|---|
| sample size | 1314 | 1077 | 859 | 1454 | 1578 | 1600 | 1510 | 1482 | 1474 |
| *Salmonella* spp. positive (%) | 29,1 | 20,2 | 17,6 | 21 | 16,3 | 13,4 | 11,3 | 7,4 | 9,4 |
| | Main serovars as a fraction of all isolates (%) | | | | | | | | |
| Paratyphi B var. Java | 15 | 11,4 | 13,9 | 33,1 | 43,2 | 53,5 | 45,6 | 58,2 | 46,8 |
| Enteritidis | 20,2 | 12,8 | 26,4 | 6,6 | 8,2 | 2,3 | 8,8 | 5,5 | 7,2 |
| Hadar | 10,1 | 6,1 | 4,5 | 3,3 | 4,2 | 0,9 | 1,8 | - | 1,4 |
| Indiana | 6,1 | 8,3 | 9,3 | 10,2 | 11,6 | 6,5 | 6,4 | 1,8 | 3,2 |
| Infantis | 9,2 | 5 | 3,6 | 6,6 | 7 | 7,9 | 11,7 | - | 11,5 |
| Virchow | 4,6 | 2,8 | 2,6 | 10,2 | 3,5 | 5,6 | 5,8 | 4,5 | 8,6 |
| 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 | 5,0 |
| Corvallis | | | | | | | | | 4,3 |
| Other types | 27 | 50 | 38,4 | 29,9 | 14,9 | 15,8 | 5,8 | 9,1 | 13,0 |

## *Salmonella* spp. in animal feeds, turkeys, horses, ducks, pigeon and reptiles

Table 16 presents the most prevalent serovars found in animal feeds from 2001 – 2005. Senftenberg Agona, Lexington, Mbandaka and Rissen are most frequently isolated. Resistance in these serovars is very uncommonly present, 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 resistance was highest in turkeys and ducks, animals with a substantial consumption of quinolones (only fluoroquinolones are licensed in Turkeys).

**Table 16. The most prevalent serovars isolated from animal feed and resistance (R%) of isolates of *Salmonella* spp. per single and or compound feed type, in 2001 – 2005. Moreover R% of Salmonella strains isolated from incidental animal sources over 2001 – 2005 are presented.**

| | | | Animal feed (or ground substance) | | | | | | | | Animals | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Serovars isolated** | | | **Fish meal (54)** | **Animal meal (50)** | **Soy (feed, (773)** | **Rapeseed (feed, (325)** | **Single feed, other (264)** | **Composite feed (111)** | **Feed 2005, (441)** | **Feed 2001-2004, (1004)** | **Turkey (41)** | **Horse (41)** | **Duck (16)** | **Pigeon (39)** | **Reptilian/Amfibian (69)** |
| Senftenberg | 196 | **Antibiotics** | **R% 2001-2005** | | | | | | **R%** | **R%** | **R% 2001-2005** | | | | |
| Agona | 175 | Amoxicillin | 0 | 1 | 3 | 3 | 5 | 1 | 0,7 | 0,9 | 29,3 | 19,5 | 25,0 | 12,8 | 1,4 |
| Lexington | 152 | Cefotaxime | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mbandaka | 148 | Imipenem | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2,4 | 0 | 0 | 0 |
| Rissen | 110 | Gentamicin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7,3 | 0 | 0 | 0 | 0 |
| Cubana | 80 | Neomycin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7,3 | 0 | 0 | 0 | 0 |
| Tennessee | 69 | Tetracycline | 0 | 1 | 14 | 4 | 10 | 9 | 0,3 | 3,6 | 19,5 | 17,1 | 12,5 | 12,8 | 2,9 |
| Anatum | 65 | Sulfamethoxazole | 0 | 0 | 2 | 1 | 1 | 1 | 0,5 | 0,2 | 15,0 | 3 | 0 | 5,3 | 0 |
| Livingstone | 64 | Trimetoprim | 0 | 1 | 3 | 1 | 1 | 3 | 0,2 | 0,8 | 2,4 | 17,1 | 0 | 0 | 0 |
| Havana | 49 | Nalidixic acid | 0 | 1 | 0 | 0 | 3 | 2 | 0,2 | 0,5 | 24,4 | 2,4 | 18,8 | 0 | 0 |
| Kentucky | 47 | Chloramphenicol | 0 | 1 | 2 | 3 | 6 | 3 | 0,7 | 1,1 | 2 | 17,1 | 6,3 | 12,8 | 1,4 |
| Oranienburg | 39 | Florfenicol | 0 | 0 | 2 | 3 | 2 | 0 | 0,2 | 0,6 | 2 | 7,3 | 0 | 12,8 | 1,4 |
| Minnesota | 34 | | | | | | | | | | | | | | |
| Montevideo | 34 | | | | | | | | | | | | | | |
| 14 main serotypes | 1262 (80%) | | | | | | | | | | | | | | |
| All serotypes | 1577 | | | | | | | | | | | | | | |

## *Campylobacter* spp.

**Highlights**

In 2005 for the first year *Campylobacter* spp. from dairy cows and veal calves were included in the surveillance. Isolates from veal calves showed the highest levels of resistance and multi drug resistance, whereas isolates from dairy cows were mostly susceptible.

Also for the first year isolates from poultry raw meat products imported from Southern America and from biologically reared poultry and pigs were included. In isolates from imported products, resistance to erythromycin, representing the first choice drug for human therapy of campylobacteriosis, occurred considerably more frequently than in isolates from Dutch poultry. Surprisingly in isolates from biological poultry and pigs resistance levels were similar to those of conventionally reared animals. Colonisation of biological animals with resistant campylobacters from the environment may be an explanation.

Both in isolates from humans and food animals the resistance levels shows a general tendency to increase, except erythromycin resistance in *C. coli* from pigs which decreased after the ban of the growth promoter tylosine in 1999.

**Table 17. MIC distribution (in %) for all *C. jejuni* (N = 122, of which 78 from broilers, 14 from dairy cows and 30 from veal calves) and *C. coli* (N = 217, of which 153 from pigs, 5 from broilers and 59 from veal calves) isolated from poultry, pig and cattle feces in The Netherlands in 2005.**

| | MIC (%) distribution (mg/L) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. jejuni (N = 122)* | **0.125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** | **1024** | **R%** |
| Amoxicillin | | | 0.8 | 6.6 | 11.5 | 38.5 | 10.7 | 2.5 | 9.8 | 2.5 | 17.2 | | | | 29.5 |
| Gentamicin | | 95.1 | 3.3 | | 0.8 | | | | | 0.8 | | | | | 1.6 |
| Neomycin | | | 85.2 | 9.8 | | | | 1.6 | 2.5 | 0.82 | | | | | 4.9 |
| Streptomycin | | | | 95.9 | 0.8 | | | | 1.6 | | | 1.6 | | | 3.3 |
| Tetracycline | 13.1 | | 34.4 | 1.6 | 0.8 | 3.3 | 7.4 | 11.5 | 7.4 | 6.6 | 13.9 | | | | 50.0 |
| Trim/sulpha | | | 3.3 | 12.3 | 42.6 | 35.2 | 2.5 | 1.6 | 0.8 | 1.6 | | | | | 2.5 |
| Sulphamethoxazole | | | | | | | 2.5 | 4.9 | 35.2 | 49.2 | 5.7 | 0.8 | 1.6 | | 1.6 |
| Ciprofloxacin | 43.4 | 9.0 | 4.9 | 2.5 | | | 7.4 | 16.4 | 16.4 | | | | | | 40.2 |
| Nalidixic acid | | | | 0.8 | 34.4 | 17.2 | 6.6 | 1.6 | | 1.6 | 24.6 | 13.1 | | | 39.3 |
| Erythromycin | | | 7.4 | 29.5 | 36.9 | 23.8 | 2.5 | | | | | | | | 2.5 |
| Chloramphenicol | | | | | 59.0 | 19.7 | 11.5 | 8.2 | 1.64 | | | | | | 1.6 |
| | **MIC (%) distribution (mg/L)** | | | | | | | | | | | | | | |
| *C. coli (N = 217)* | **0.125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** | **1024** | **R%** |
| Amoxicillin | | 2.8 | 5.5 | 13.8 | 19.8 | 33.2 | 11.5 | | 6.0 | 0.9 | 6.5 | | | | 13.4 |
| Gentamicin | | 65.9 | 31.8 | 1.4 | | | | | | 0.9 | | | | | 0.9 |
| Neomycin | | | 34.1 | 56.2 | 1.4 | | | 0.5 | 1.8 | 2.3 | 3.7 | | | | 8.3 |
| Streptomycin | | | | 8.8 | 10.1 | 1.8 | 0.5 | 0.9 | 41.9 | 21.2 | 1.8 | 12.9 | | | 79.3 |
| Tetracycline | 0.5 | | 7.4 | 1.8 | 1.4 | 0.5 | 1.8 | 14.3 | 19.8 | 24.9 | 27.6 | | | | 88.9 |
| Trim/sulpha | | 4.1 | 11.1 | 13.4 | 13.8 | 5.5 | 0.9 | 12.0 | 35.9 | 0.5 | 2.7 | | | | 57.6 |
| Sulphamethoxazole | | | | | | | 10.6 | 15.7 | 8.8 | 12.9 | 0.5 | 4.6 | 38.2 | 8.8 | 65.0 |
| Ciprofloxacin | 41.9 | 26.3 | 5.1 | | | | 1.4 | 15.7 | 9.7 | | | | | | 26.7 |
| Nalidixic acid | | | | 0.5 | 12.0 | 38.7 | 21.2 | 0.9 | | 1.8 | 19.8 | 5.1 | | | 26.7 |
| Erythromycin | | | 2.8 | 4.1 | 9.2 | 43.3 | 26.3 | 6.5 | 0.5 | 0.5 | 6.9 | | | | 7.8 |
| Chloramphenicol | | | | | 13.4 | 45.6 | 35.5 | 5.5 | | | | | | | 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 breakpoints.

Table 17 presents the MIC-distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated from broilers and slaughter pigs in 2005. The MIC-distributions are bimodal for most antibiotics and the epidemiological cut off values used, adequately distinguish resistant from susceptible populations for most antibiotics used in the test panels. However for sulphamethoxazole alone or in combination with trimethoprim the cut off values are not entirely adequate and as a result the resistance percentages for these antibiotics are overestimated.

Table 18 shows resistance percentages for *Campylobacter* spp. for the different sources. In *C. jejuni* from poultry the resistance levels in isolates from broiler faeces, from poultry raw meat products sampled by the Dutch Food Safety Authority at retail, and from biologically reared poultry are very similar. This was expected for isolates from broiler faeces and poultry raw meat products because they should represent the same bacterial populations. For isolates from biologically reared poultry this was not anticipated. In the Netherlands the number of biological poultry farms is very limited (N = 9) and all of them were included in this surveillance. At these farms five pooled fecal samples were taken from fresh droppings at each farm. All farms were positive for *C. jejuni* and/or *C. coli*. From the 45 pooled fecal samples, 29 were positive for *C. jejuni* and 3 for *C. coli* . At these farms a restrictive antibiotics use policy exists. Moreover, the animals are reared up to 70 days, which is approximately twice as long as broilers. Therefore lower resistance levels were expected. However, poultry is normally contaminated with *Campylobacter* spp. from the environment. In the Dutch environment resistant *Campylobacter* spp. originating from broilers or other intensively reared poultry, may be very commonly present and may have constituted a source for colonisation of biological poultry as well.
*C. jejuni* from imported products demonstrated clear differences in its resistance phenotype compared to the isolates from Dutch origin. Resistance to aminoglycosides and erythromycin occurred more frequently, whereas resistance levels for amoxicillin and tetracycline were lower.

Molecular analysis of all isolates that were classified to be resistant to erythromycin according to the epidemiological cut off value used, demonstrated that only in the isolates from imported poultry product the typical mutations were demonstrated. These isolates all demonstrated high level resistance (MIC $\geq$ 64 mg/L). In isolates with MICs of 8 mg/L no mutations were detected.

The resistance percentages of *C. coli* from poultry and products were also very similar, except the resistance levels for trimethoprim/sulpha This may be an artefact as a result of differences in interpretations of the raw data between the two laboratories involved. For sulphonamides and trimethoprim this can be very difficult and is a known source of variation in MIC data.
*C. coli* from imported poultry products showed high levels of neomycin and erythromycin resistance, similar as was observed in *C. jejuni* and lower levels of tetracycline resistance.
In *C. coli* from pigs the resistance levels in isolates from biological pigs were slightly lower than those from conventional pigs. For biological pigs 30 farms were included in the surveillance and from each farm 5 pooled faecal samples were taken. Twenty-five (83%) were positive for Campylobacter and from 82 samples *C. coli* was isolated.
In *Campylobacter* spp. from veal calves highest resistance levels were observed for gentamicin, neomycin, streptomycin, tetracycline, ciprofloxacin, nalidixic acid and erythromycin in *C. jejuni*.
Most isolates from dairy cattle were susceptible to all antibiotics.

For human infections poultry products are considered to be the most important animal source. For that reason in Dutch poultry production active control strategies on food borne pathogens are undertaken. Isolates from imported poultry products show higher levels of resistance and more resistance of public health concern.

In Campylobacter multi drug resistance is highest in *C. coli* compared to *C. jejuni* and highest in veal calves compared to poultry and pigs (Fig. 15). This reflects the differences in occurrence of the two C. spp. In the animal categories and the difference in selection pressure as a result of antibiotic usage in these animal categories.

Figure 16 shows that in humans *Campylobacter* spp. resistance to fluoroquinolones (data are based on disk diffusion tests for norfloxacin, ofloxacin and ciprofloxacin) slowly increased in the last decade, to more than 36% in 2005. Also resistance to tetracyclines increased in 2005 to 25%.Resistance to macrolides remained stable at a very low level.

**Figure 15. Percentages of *Campylobacter* strains isolated from faecal samples fully susceptible, resistant to one antimicrobial class up to a maximum of nine different classes in isolates from dairy cattle broilers, slaughter pigs and veal calves in The Netherlands in 2005.**

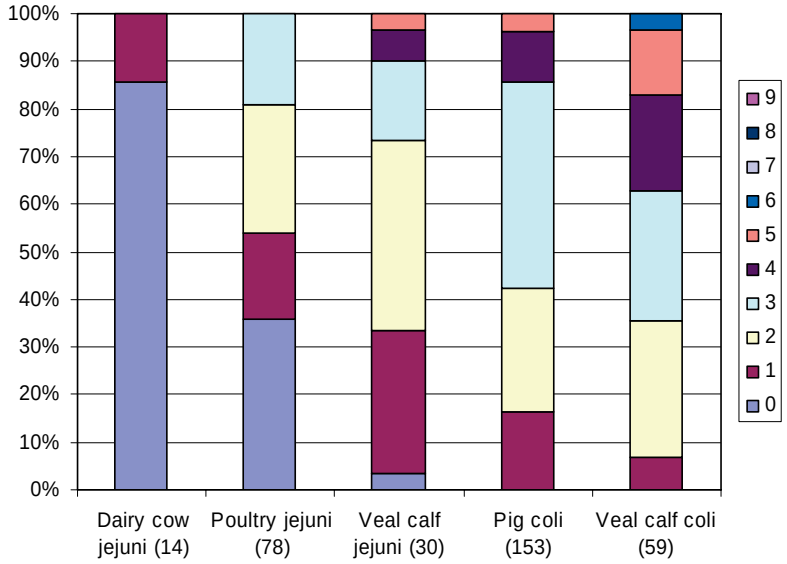



**Figure 16. Trends in resistance (%) of *Campylobacter* spp. isolated from humans isolated between 1992 and 2005 at the regional Public Health Laboratories (PHLS) of Arnhem and Heerlen covering 990.000 inhabitants. The dotted line represents data from the national surveillance in 2002 - 2005; annually the average number of strains tested was approximately 2400, ranging from 1900 – 2900.**

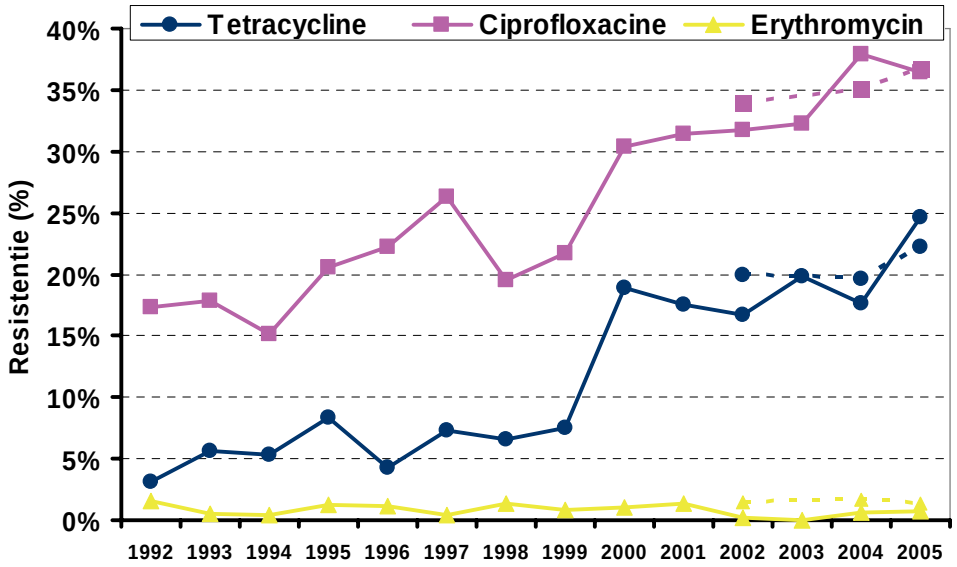
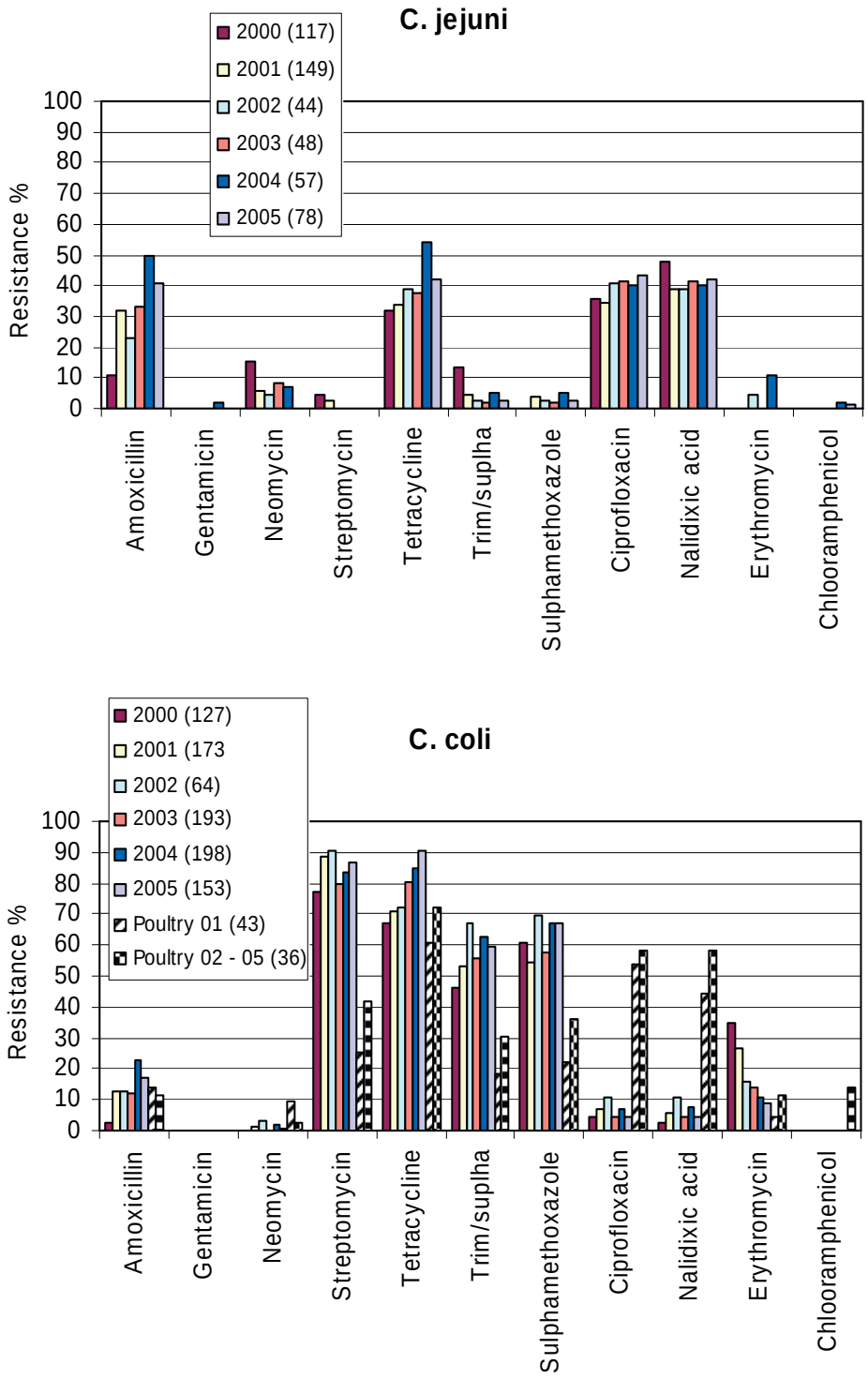


**Table 18. Resistance percentages of *C. jejuni* and *C. coli* isolated from broilers, raw meat products, imported poultry raw meat products, slaughter pigs, veal calves and dairy cows in 2005**

| | Broilers | Poultry products | Import poultry products | Biological poultry | Broilers | Poultry products | Import poultry products | Pigs | Biological pigs | Veal calves | Veal calves | Dairy cows |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C. jejuni | C. jejuni | C. jejuni | C. jejuni | C. coli | C. coli | C. coli | C. coli | C. coli | C. jejuni | C. coli | C. jejuni |
| | (78) | (70) | (30) | (29) | (5) | (37) | (30) | (153) | (82) | (30) | (59) | (14) |
| Amoxicillin | 41 | 44.3 | 13.9 | 48.3 | 20 | 32.4 | 36.7 | 17 | 7.3 | 10 | 3.4 | 7.1 |
| Gentamicin | 0 | 0 | 5.6 | 0 | 0 | 0 | 0 | 0 | 0 | 6.7 | 3.4 | 0 |
| Neomycin | 0 | 1.4 | 33.3 | 6.9 | 0 | 2.7 | 30 | 0.7 | 0 | 20 | 28.8 | 0 |
| Streptomycin | 0 | 1.4 | 8.3 | 0 | 20 | 32.4 | 3.3 | 86.9 | 76.8 | 13.3 | 64.4 | 0 |
| Tetracycline | 42.3 | 50 | 36.1 | 41.4 | 60 | 64.9 | 33.3 | 86.3 | 47.6 | 90 | 98.3 | 7.1 |
| Trim/sulpha | 1.3 | 1.4 | 0 | 0 | 0 | 35.1 | 26.7 | 59.5 | 24.4 | 6.7 | 57.6 | 0 |
| Sulphamethoxazole | 2.6 | 1.4 | 0 | 0 | 20 | 54.1 | 30 | 54.9 | 18.3 | 0 | 30.5 | 0 |
| Ciprofloxacin | 43.6 | 47.1 | 38.9 | 55.2 | 60 | 56.8 | 46.7 | 4.6 | 4.9 | 50 | 81.4 | 0 |
| Nalidixic acid | 42.3 | 47.1 | 38.9 | 55.2 | 60 | 56.8 | 46.7 | 4.6 | 4.9 | 50 | 81.4 | 0 |
| Erythromycin | 0 | 2.9 | 19.4 | 0 | 0 | 8.1 | 20 | 9.2 | 11 | 10 | 5.1 | 0 |
| Chloramphenicol | 1.3 | 0 | 5.6 | 0 | 0 | 0 | 0 | 0 | 0 | 3.3 | 0 | 0 |

**Figure 17. Trends in resistance (%) of *C. jejuni* isolated from broilers and *C. coli* isolated from slaughter pigs and broilers (grey striped bars), from 2000 - 2005**



A tendency to increase in resistance can be observed both in isolates from poultry and from pigs which may be explained by the high (increasing) usage of antibiotics in The Netherlands. Resistance to nalidixic acid and ciprofloxacin is stable in poultry (Fig. 17). In pigs in *C. coli* resistance to erythromycin has decreased after the ban of macrolides as growth promoter in 1999.

**Table 19. Domestically acquired and travel related resistance in *C. jejuni* and *C. coli* isolated from humans in 2005 from all 16 PHLS covering > 50% of the Dutch population.**

| | **Domestically acquired** | | | | **Travel-related** | | | |
|---|---|---|---|---|---|---|---|---|
| | ***C. jejuni*** | | ***C. coli*** | | ***C. jejuni*** | | ***C. coli*** | |
| | N | R% | N | R% | N | R% | N | R% |
| Fluoroquinolone | 2456 | 35,1% | 161 | 36,6% | 153 | 37,3% | 22 | 54,5% |
| Tetracycline | 1800 | 20,8% | 148 | 20,9% | 94 | 33,0% | 19 | 15,8% |
| Erythromycin | 2095 | 1,5% | 153 | 4,6% | 128 | 2,3% | 21 | 0,0% |

Table 19 shows that in travel-related infections fluoroquinolone resistance occurred more frequently than in isolates from domestically acquired infections, for tetracycline this difference was also observed for *C. jejuni* only. It is surprising that erythromycin resistance did not occur in travel related *C. coli.*

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

In 2005, 42 isolates of notifiable Shi-toxin producing *E. coli* O157 (STEC) were sent to RIVM for routine typing. 24 specimens were taken from human faeces and 11 from veal calves and dairy cattle in an attempt to trace a human clinical infection. In humans in 2005 an explosion of 25 cases occurred that were infected with one clonal outbreak strain, as was found at RIVM-Bilthoven by pulsed field gel electrophoresis. This clone was resistant to sulphamethoxazole and trimethoprim. In the analysis of the data only one isolate representing this clone was included.
Resistance in *E. coli* O157 is rarely present and limited to isolates from sporadic cases. Next to the outbreak clone resistance only occurred in two isolates from humans. In the cattle isolates one was resistant to amoxicillin, sulpha and trimethoprim, the other to tetracycline, sulpha, florfenicol and chloramphenicol. Florfenicol resistance is rare in *E. coli*

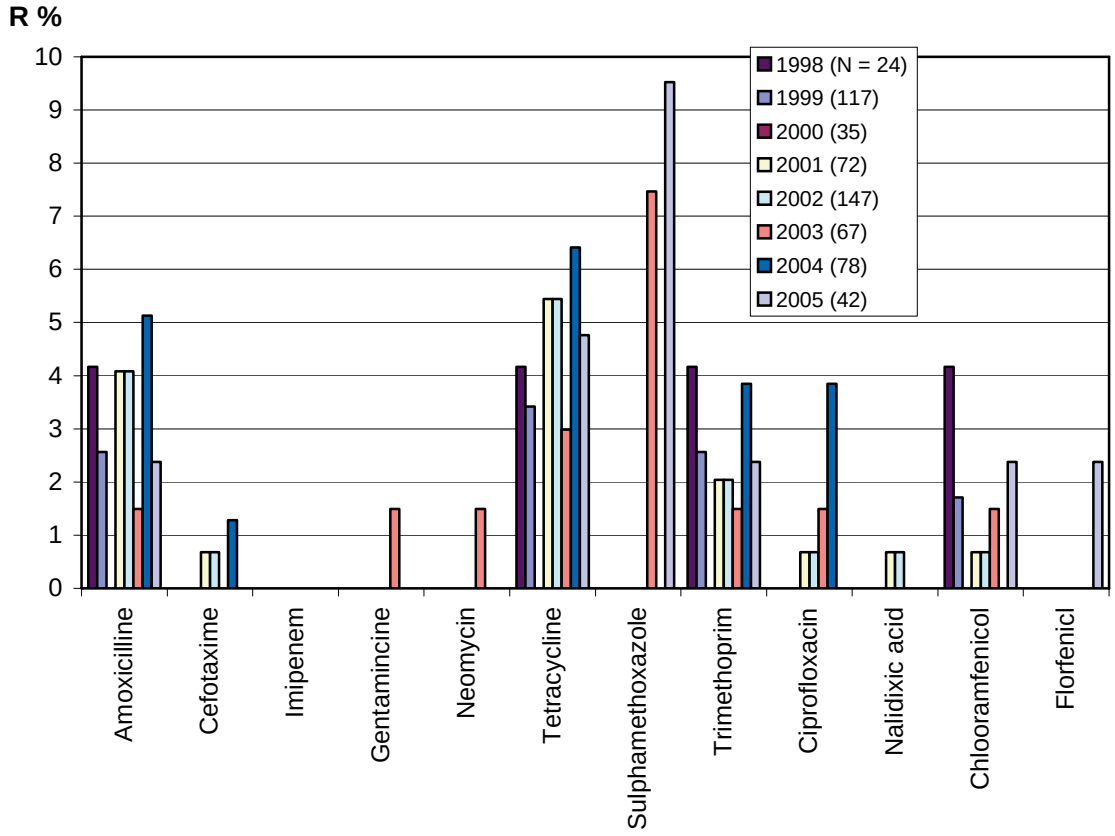
**Highlights**
The resistance levels for *E. coli* O157 were low and limited to a small number of individual isolates. Two isolates from cattle were multi-drug resistant. In 2005 an outbreak occurred with a cluster of 25 human cases. This outbreak clone was resistant to trimethoprim and sulphamethoxazole

**Table 20. MIC distribution (in %) for *E. coli* O157 isolated in The Netherlands in 2005 from human (N = 31) and cattle faeces (N = 11)**

| | MIC (%) distribution (mg/L) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Human (31)** | **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%** |
| Amoxicillin | | | | | | | | 3,2 | 96,8 | | | | | | | | | | 0 |
| Cefotaxim | | | | 100 | | | | | | | | | | | | | | | 0 |
| Ceftazidime | | | | 80,6 | 19,4 | | | | | | | | | | | | | | 0 |
| Gentamicin | | | | | 22,6 | 64,5 | | | | | | | | | | | | | 0 |
| Neomycin | | | | | | | 96,8 | | | | | | | | | | | | 0 |
| Tetracycline | | | | | | | | 96,8 | | | | | | 3,2 | | | | | 3,2 |
| Sulphamethoxazole | | | | | | | | | | 93,5 | | | | | | | | 6,5 | 6,5 |
| Trimethoprim | | | | | | 100,0 | | | | | | | | | | | | | 0 |
| Ciprofloxacin | | | 100 | | | | | | | | | | | | | | | | 0 |
| Nalidixic acid | | | | | | | | 74,2 | 25,8 | | | | | | | | | | 0 |
| Chloramphenicol | | | | | | | | | 3,2 | 80,6 | 16,1 | | | | | | | | 0 |
| Florfenicol | | | | | | | | | 12,9 | 87,1 | | | | | | | | | 0 |
| | **MIC (%) distribution (mg/L)** | | | | | | | | | | | | | | | | | | |
| **Cattle (11)** | **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%** |
| Amoxicillin | | | | | | | | | 81,8 | 9,1 | | | | 9,1 | | | | | 9,1 |
| Cefotaxim | | | | 100 | | | | | | | | | | | | | | | 0 |
| Ceftazidime | | | | 72,7 | 27,3 | | | | | | | | | | | | | | 0 |
| Gentamicin | | | | | 36,4 | 63,6 | | | | | | | | | | | | | 0 |
| Neomycin | | | | | | | 90,9 | 9,1 | | | | | | | | | | | 0 |
| Tetracycline | | | | | | | 9,1 | 81,8 | | | | | | 9,1 | | | | | 9,1 |
| Sulphamethoxazole | | | | | | | | | | 81,8 | | | | | | | | 18,2 | 18,2 |
| Trimethoprim | | | | | | 90,9 | | | | | | | | 9,1 | | | | | 9,1 |
| Ciprofloxacin | | | 100 | | | | | | | | | | | | | | | | 0 |
| Nalidixic acid | | | | | | | | 72,7 | 27,3 | | | | | | | | | | 0 |
| Chloramphenicol | | | | | | | | | | 90,9 | | | | | 9,1 | | | | 9,1 |
| Florfenicol | | | | | | | | | 9,1 | 81,8 | | | | | 9,1 | | | | 9,1 |

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 breakpoint.

**Figure 18. Trends in resistance percentages of *E. coli* O157 (STEC) isolated in The Netherlands from 1998 - 2005**




Resistance data from 1998 to 2005 demonstrate the absence of clear trends. Throughout the years the levels showed a lot of variation and only incidentally resistance to modern antibiotics like cefotaxime, gentamicin or nalidixic acid was observed.

## Food-borne commensal 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 or growth promoters 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. $10^6$ cfu/g faeces *E. coli* in its gut, 1% would be app. $10^4$cfu/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**
In isolates from intensively reared broilers and veal calves, and to a slightly lesser extend slaughter pigs, resistance levels are very high and show a tendency to increase over time. In dairy cows resistance is only rarely present.
Multi drug resistance shows a similar increasing trend, with alarmingly high levels of multi drug resistant isolates in broilers and veal calves.
The occurrence of extended spectrum beta-lactamases increased substantially in broilers from 9.7% in 2004 to 14.1% in 2005, this in spite of the fact that cephalosporins are not used in these animals. This means that linkage of resistance genes and co-selection by usage of other antibiotics probably has been the main cause for the observed increase.
Resistance in isolates from imported poultry products from Southern America were higher while resistance in isolates from biological animals was lower compared to those from Dutch meat products or conventional Dutch animals.

In isolates from all animal species, the older classes of antibiotics, amoxicillin, tetracycline, trimethoprim and sulphamethoxazole showed the highest resistance levels (table 21). Moreover, the resistance levels in broilers and veal calves were higher than those in pigs and dairy cattle.
In broilers resistance to ciprofloxacin and nalidixic acid was very high (> 50%). High level ciprofloxacin resistance occurred in 2.6% of the isolates. Although the resistance levels for nalidixic acid from 1998 to 2005 show a certain annual variation, they show a tendency to increase (Fig. 20).
In broilers resistance to cefotaxime and ceftazidime, indicative of the presence of extended spectrum beta-lactamases (ESBLs), has increased from 9.7% in 2004 to >14% in 2005. A genetic analysis of isolates from Dutch broilers conducted at VLA-Weighbridge, revealed that CTX-M type ESBLs (1 and 2) are predominant in these isolates. Also CMY-2 and incidental TEM and SHV variants are present. Because the sample of 300 *E. coli* strains is randomly isolated from caecal samples at slaughter, it strongly indicates that plasmid mediated ESBLs and AmpC-type beta-lactamases are very commonly present in the gastro-intestinal flora of broilers, which may act as a biological reservoir for transmission to other pathogens, like *Salmonella*. In *Salmonella* Paratyphi B var. Java which is commonly present in broilers in The Netherlands and in one *E. coli* O157 from broilers, the same ESBLs (CTX-M2) were detected indicative of horizontal transfer from *E. coli.*

**Table 21. MIC distributions (in %) for E. coli isolated as indicator organism from intestines of slaughter pigs (N = 299), broiler chickens (N = 300), veal calves (N = 165) and dairy cows (N = 139) in The Netherlands in 2005.**

| Slaughter pigs (299) | 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%** |
| Amoxicillin | | | | | | 0.3 | 2.7 | 7.7 | 43.5 | 15.4 | | 0.3 | | 30.1 | | | | | 30.4 |
| Cefotaxim | | | | 97.7 | 1.7 | 0.3 | | 0.3 | | | | | | | | | | | 0.7 |
| Ceftazidime | | | | 76.9 | 20.2 | 2.5 | | | 0.4 | | | | | | | | | | 0.4 |
| Gentamicin | | | | | 8.4 | 68.9 | 18.7 | 3.0 | | 0.7 | | | 0.3 | | | | | | 1.0 |
| Neomycin | | | | | | | 77.6 | 14.4 | 3.3 | 0.3 | | 2.0 | 1.7 | 0.7 | | | | | 4.3 |
| Tetracycline | | | | | | | 9.4 | 24.1 | 4.3 | 0.3 | 1.0 | 2.7 | 28.1 | 30.1 | | | | | 61.9 |
| Sulphamethoxazole | | | | | | | | | | 48.2 | 0.3 | 0.3 | | | | | 0.7 | 50.5 | 51.2 |
| Trimethoprim | | | | | | 56.2 | 2.0 | 0.3 | | | | | | 41.5 | | | | | 41.5 |
| Ciprofloxacin | | | 100 | | | | | | | | | | | | | | | | 0 |
| Nalidixic acid | | | | | | | | 70.9 | 28.1 | 1.0 | | | | | | | | | 0 |
| Chloramphenicol | | | | | | | | | 6.4 | 77.9 | 7.0 | 1.7 | 4.3 | 0.3 | 2.3 | | | | 8.7 |
| Florfenicol | | | | | | | | 1.3 | 15.1 | 75.6 | 8.0 | | | | | | | | 0 |
| **Broilers (300)** | **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%** |
| Amoxicillin | | | | | | | 1.3 | 5.6 | 20.7 | 8.9 | 0.3 | | 0.3 | 62.8 | | | | | 63.5 |
| Cefotaxime | | | | 85.5 | 0.3 | | | 0.7 | 0.7 | 1.3 | 2.6 | 8.9 | | | | | | | 14.1 |
| Ceftazidime | | | | 52.9 | 29.1 | 3.3 | 3.3 | 2.9 | 4.5 | 2.0 | 1.6 | 0.4 | | | | | | | 14.8 |
| Gentamicin | | | | | 6.9 | 69.4 | 17.8 | 2.0 | | 0.7 | 1.6 | 1.3 | 0.3 | | | | | | 3.9 |
| Neomycin | | | | | | | 69.4 | 17.1 | 2.0 | 0.3 | 0.7 | 4.9 | 2.3 | 2.6 | 0.7 | | | | 11.2 |
| Tetracycline | | | | | | | 5.6 | 22.4 | 11.2 | | | 1.3 | 29.3 | 30.3 | | | | | 60.9 |
| Sulphamethoxazole | | | | | | | | | | 28.3 | | | | | | | | 71.4 | 71.6 |
| Trimethoprim | | | | | | 34.5 | 2.3 | | | | | | | 63.2 | | | | | 63.2 |
| Ciprofloxacin | | | 49.0 | 9.9 | 22.4 | 13.5 | 2.0 | 0.3 | 1.0 | 1.6 | | | | | | | | | 50.8 |
| Nalidixic acid | | | | | | | | 36.5 | 11.5 | | | 2.3 | 6.9 | 18.8 | 24.0 | | | | 52.0 |
| Chloramphenicol | | | | | | | | | 2.3 | 65.1 | 14.5 | | 1.0 | 0.7 | 16.4 | | | | 18.1 |
| Florfenicol | | | | | | | | | 15.1 | 71.1 | 12.8 | 0.7 | 0.3 | | | | | | 1.0 |
| **Veal calves (165)** | **15** | **3** | **6** | **0.125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** | **1024** | **2048** | **R%** |
| Amoxicillin | | | | | | | 2.4 | 6.7 | 33.9 | 8.5 | | | | 48.5 | | | | | 48.5 |
| Cefotaxime | | | | 91.5 | 5.5 | 0.6 | 1.2 | 0.6 | | | | 0.6 | | | | | | | 3.0 |
| Ceftazidime | | | | 55.2 | 38.8 | 5.5 | | | | | 0.6 | | | | | | | | 0.6 |
| Gentamicin | | | | | 3.0 | 55.8 | 23.0 | 5.5 | 1.2 | 0.6 | 4.8 | 3.0 | 3.0 | | | | | | 12.7 |
| Neomycin | | | | | | | 55.8 | 15.2 | 1.2 | 0.6 | 0.6 | 3.0 | 7.9 | 7.3 | 8.5 | | | | 27.3 |
| Tetracycline | | | | | | | 0.6 | 13.9 | 3.0 | | 0.6 | 2.4 | 17.6 | 61.8 | | | | | 82.4 |
| Sulphamethoxazole | | | | | | | | | | 45.5 | | | | | | | | 54.5 | 54.5 |
| Trimethoprim | | | | | | 53.9 | 1.8 | | | | | | | 44.2 | | | | | 44.2 |
| Ciprofloxacin | | | 74.5 | 3.0 | 6.1 | 4.2 | 0.6 | 1.2 | | 0.6 | 9.7 | | | | | | | | 25.5 |
| Nalidixic acid | | | | | | | | 47.3 | 23.6 | 3.0 | | 1.8 | 1.8 | 6.7 | 15.8 | | | | 26.1 |
| Chloramphenicol | | | | | | | | | 4.8 | 47.9 | 13.9 | 2.4 | 1.8 | 4.2 | 24.8 | | | | 33.3 |
| Florfenicol | | | | | | | | | 13.3 | 51.5 | 17.0 | 5.5 | 1.8 | 1.8 | 9.1 | | | | 18.2 |
| **Dairy cows (139)** | **15** | **3** | **6** | **0.125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** | **1024** | **2048** | **R%** |
| Amoxicillin | | | | | | | 2.9 | 8.6 | 59.7 | 28.8 | | | | | | | | | 0 |
| Cefotaxim | | | | 98.6 | 1.4 | | | | | | | | | | | | | | 0 |
| Ceftazidime | | | | 76.3 | 22.3 | 1.4 | | | | | | | | | | | | | 0 |
| Gentamicin | | | | | 4.3 | 78.4 | 12.9 | 3.6 | 0.7 | | | | | | | | | | 0.7 |
| Neomycin | | | | | | | 86.3 | 11.5 | 2.2 | | | | | | | | | | 0 |
| Tetracycline | | | | | | | 16.5 | 66.2 | 16.5 | | | | 0.7 | | | | | | 0.7 |
| Sulphamethoxazole | | | | | | | | | | 98.6 | 0.7 | | | | | | | 0.7 | 0.7 |
| Trimethoprim | | | | | | 98.6 | 0.7 | | | | | | | 0.7 | | | | | 0.7 |
| Ciprofloxacin | | | 100 | | | | | | | | | | | | | | | | 0 |
| Nalidixic acid | | | | | | | | 69.8 | 29.5 | 0.7 | | | | | | | | | 0 |
| Chloramphenicol | | | | | | | | | 4.3 | 89.9 | 5.8 | | | | | | | | 0 |
| Florfenicol | | | | | | | | | 11.5 | 84.9 | 3.6 | | | | | | | | 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 breakpoints.

This phenomenon is intriguing because cephalosporins are not used in poultry, therefore other selective determinants must exist. ESBLs are often located on integrons and plasmids linked to other resistance genes. These genes encode for resistance to a.o. amoxicillin, chloramphenicol, aminoglycosides, trimethoprim and sulphonamides. Except chloramphenicol, these antimicrobial classes are often used in broilers and therefore may co-select for ESBLs. Recently linkage of ESBLs to quinolone-resistance genes (*qnr*) was described on integrons and plasmids, this means that usage of quinolone may also have contributed to this co-selection. A genetic analysis of these isolates is currently conducted at CIDC.

Isolates from veal calves demonstrated a different quinolone-resistance profile than those from other animals. More than 10% of the isolates were high level ciprofloxacin resistant. This may reflect the selection pressure of quinolone (both oral and intramuscular or subcutaneous usage of fluoroquinolones and oral usage of flumequine) usage in veal calf industry.
Isolates from dairy cattle were almost always susceptible to all antibiotics included in the test panel.

Multi drug resistance (MDR) has increased substantially in all major food animal species (Fig. 19). In broilers in 2005 app. 60% of the isolates were resistant to three antibiotic classes and app. 20% resistant to five classes. In pigs the same increase was observed but MDR was present at lower levels; in 2005 40% of the isolates was resistant to 3 antibiotic classes.
In veal calves the level of MDR is similar to the level in broilers. However, the proportion of isolates resistant to 5 or more classes is much higher than in other food animals. In de randomly selected *E. coli* isolates from veal calves one isolate was resistant to 9 different antibiotic classes. Because this strains was isolated from randomly sampled feces from veal calves (N = 165) and one sample represented one flock of animals, this indicates that these type of MDR isolates are commonly present in veal calves.
The evolution of MDR in food animals as presented in Figure 19 is a worrisome situation. It demonstrates that in these animals an environment is created where MDR strains of all kind of species of microorganisms can survive and multiply. Examples of clinical relevance are ESBL-, and integron positive *Enterobacteriaceae* and MRSA.

The resistance levels show a tendency to increase in all animal species in 2005 (Fig. 20). In broilers an increase can be observed for amoxicillin, cefotaxime, trimethoprim, the quinolones and chloramphenicol. In slaughter pigs resistance to amoxicillin increased, while resistance to tetracycline and trimethoprim seems to have reached a high steady state level after an increase in previous years. In veal calves an increase can be observed since the end of the nineties for most antibiotics except trimethoprim. In veal calves chloramphenicol resistance seems to be stable while florfenicol resistance increases, this indicates a shift in the occurrence of chloramphenicol acetyltransferase (CAT) to the FloR-gene.

**Figure 19. 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.**

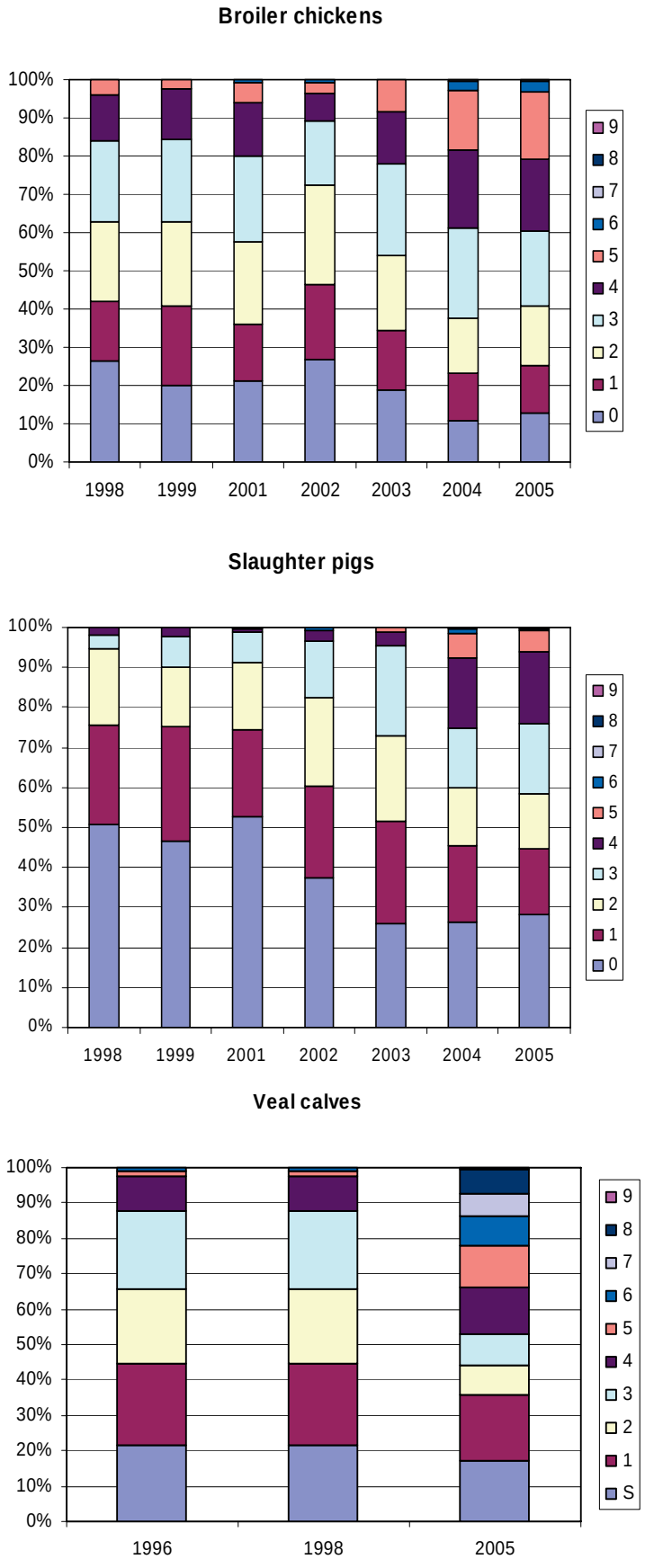
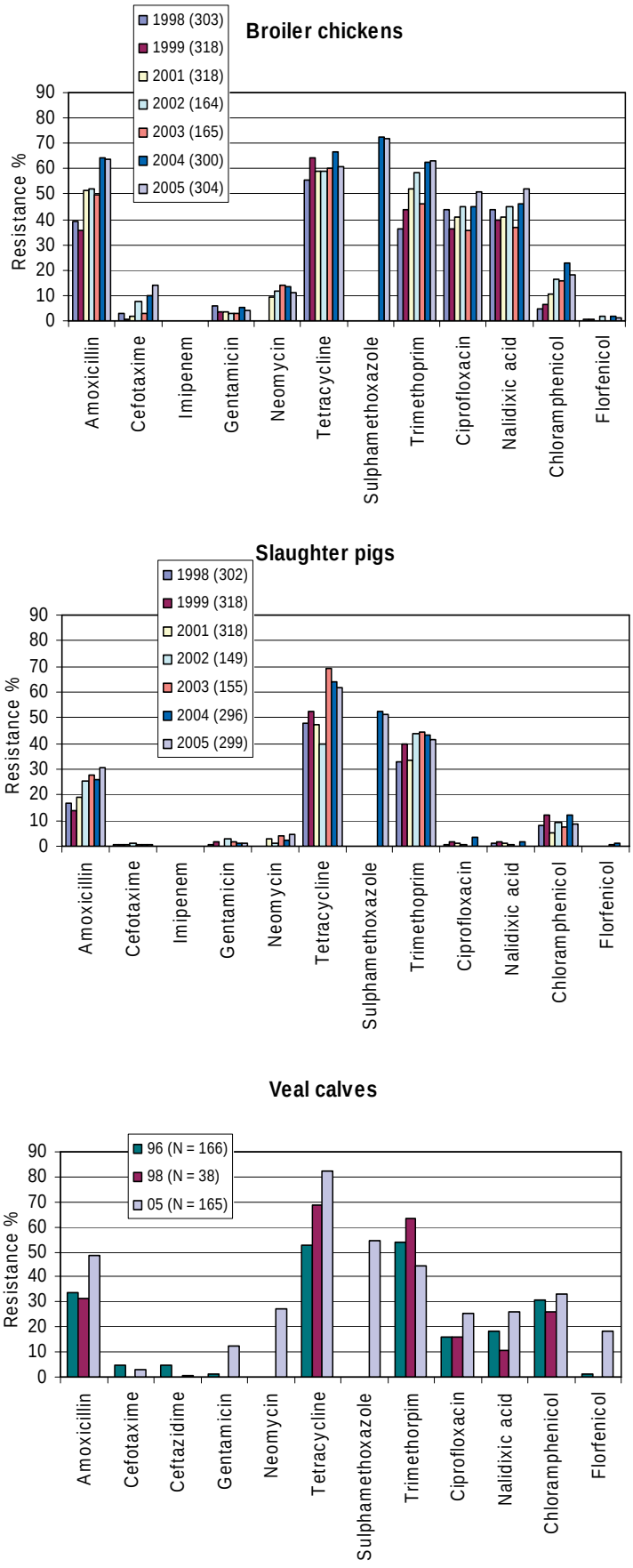


**Figure 20. Trends in resistance (in%) of *E. coli* isolated from slaughter pigs and broilers in The Netherlands from 1998 - 2005**



## *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 2005. Apart from imported poultry, samples are from products from animals reared in the Netherlands, i.e. poultry, biologically reared poultry, pork, biologically reared pigs, beef and lamb.**

| | Poultry (115) | Poultry import (110) | Biol poultry (45) | Pork (13) | Biol pork (155) | Beef (34) | Lamb (6) |
|---|---|---|---|---|---|---|---|
| Amoxicillin | 53.9 | 33.7 | 17.8 | 30.8 | 16.1 | 11.8 | 0 |
| Cefotaxim | 11.3 | 4.0 | 0 | 0 | 1.3 | 2.9 | 0 |
| Imipenem | 0 | 0 | - | 7.7 | - | 2.9 | 0 |
| Gentamicin | 4.3 | 19.8 | 0 | 0 | 3.7 | 0 | 0 |
| Neomycin | 12.2 | 23.8 | 0 | 0 | 2.6 | 0 | 0 |
| Tetracycline | 47.0 | 64.4 | 44.4 | 46.2 | 38.1 | 11.8 | 0 |
| Sulphamethoxazole | 49.6 | 59.4 | 13.3 | 7.7 | 27.1 | 14.7 | 0 |
| Trimethoprim | 42.6 | 35.6 | 11.1 | 7.7 | 16.8 | 11.8 | 0 |
| Ciprofloxacin | 29.6 | 67.3 | 6.7 | 0 | 0 | 8.8 | 0 |
| Nalidixic acid | 33.0 | 70.3 | 4.4 | 0 | 0 | 2.9 | 0 |
| Chloramphenicol | 7.8 | 12.9 | 2.2 | 0 | 5.8 | 2.9 | 0 |
| Florfenicol | 0 | 2.0 | 0 | 0 | 1.3 | 0 | 0 |

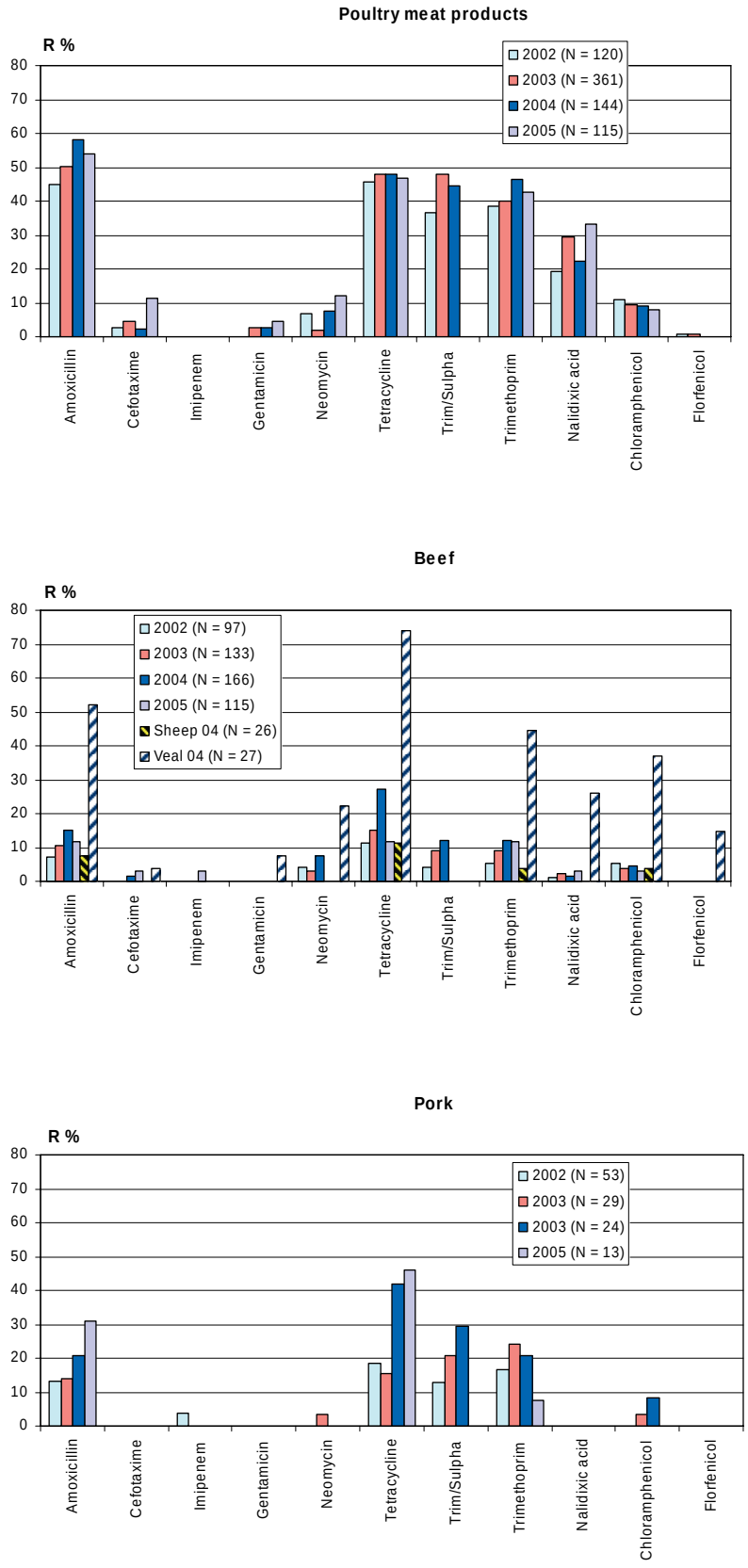
Resistance percentages of *E. coli* strains isolated from poultry products sampled at retail in the Netherlands were similar to those isolated as indicator organisms from Dutch broilers (Tables 21 and 22). However, resistance to the quinolones was substantially lower in poultry raw meat products. Highest resistance was observed in isolates from poultry raw meat product imported from Southern America and lowest resistance was observed in isolates from biological animals.
The difference observed reflects the differences in selection effect of antibiotic usage as a result of different usage practices, but consumption data to support this are lacking.

The number of strains isolated from pork are too small to conclude on differences with isolates from pig faeces. Resistance levels in isolates from biological pigs are lower than those from slaughter pigs (Table 22).

Figure 21 shows trends in resistances in the different meat products. Although the resistance percentages show a general tendency to increase, these data have to be interpreted carefully. The observed tendency may be a normal variation due to sampling methods used and not reflect a true increase because of the relatively low numbers.

Highest resistance levels are observed in isolates from veal calves.

**Figure 21. Trends in resistance (in%) of *E. coli* isolated from raw poutry meat products, beef, veal, sheep and pork, in The Netherlands from 1998 - 2005**



## *Enterococcus faecium, Enterococcus faecalis*

**Highlights**
In 2005 for the first time since 1997 cattle (dairy cows and veal calves) were included in the monitoring programme. In isolates from dairy cows the lowest resistance levels were observed as was expected. In these animals the main usage of antibiotics is in local treatment of mastitis or intra-uterine infections. In the major food producing animals (broilers, slaughter pigs and veal calves) resistance occurred more frequently.
Amoxicillin resistance was only observed at relatively low levels in *E. faecium* isolated from cattle and broilers. In veal calves amoxicillin resistance substantially increased from 0% in 1997 to 10.7% in 2006, in broilers the levels were stable.
For the first year a few linezolid resistant strains were detected in veal calves and in poultry products, this in spite of the fact linezolid, a member of the oxazolidone class, is not used in animals at all.
In 2005 high level ciprofloxacin resistant *E. faecalis* isolates were observed (MIC ≥ 16 mg/L). These strains were isolated from veal calves and broilers, the animal species in which quinolones are predominantly used.

In 2005 besides slaughter pigs and broilers, for the first time since 1997, cattle (dairy cows and veal calves) were included in the programme. To reduce the number of tables, the quantitative information on the MIC-distributions are summarized for *E. faecalis* and *E. faecium* (Table 23). In Table 24 the calculated resistance percentages are presented for each bacterial species and specified by the different animal sources.

In strains from all sources, resistance levels were higher in *E. faecium* compared to *E. faecalis.* However, for individual drugs the opposite occurs and resistance was more common in *E. faecalis* (tetracycline, erythromycin and high level streptomycin resistance)(Tables 23 and 24, Fig. 22).

Amoxicillin resistance was only observed at relatively low levels in *E. faecium* isolated from cattle and broilers. In veal calves amoxicillin resistance substantially increased from 0% in 1997 to 10.7% in 2006, in broilers the levels were stable.

One linezolid resistant *E. faecium* strains was isolated from veal calves (MIC 8 mg/L). Linezolid resistance has been described to occur in enterococci with similar or higher MIC levels (16 – 64 mg/L) have been described in the UK in 2002 (Auckland *et al.*) en in Greece in 2004 (Bersos *et al.*). The described resistance mechanism is a single point mutation in the 23S ribosomal DNA. A molecular analysis of the Dutch veal calf will be conducted.

In 2005 high level ciprofloxacin resistant *E. faecalis* isolates were observed (MIC ≥ 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 all animal species, but the MICs were lower compared to *E. faecalis*.

**Table 23. MIC distributions (In %) for *E. faecalis* (N = 231) and *E. faecium* (N = 443) isolated in food producing animals in The Netherlands in 2005**

.

| | MIC (%) distribution (mg/L) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***E. faecalis*** **(N = 231)** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** | **1024** | **2048** | **R (%)** |
| Amoxicillin | | | 98.3 | 1.7 | | | | | | | | | | | 0 |
| Linezolid | | | 8.7 | 90.5 | 0.9 | | | | | | | | | | 0 |
| Tetracycline | | 21.2 | 4.8 | 0.9 | | | 0.9 | 9.1 | 24.7 | 37.2 | 1.3 | | | | 73.2 |
| Erythromycin | | | 21.6 | 12.6 | 9.1 | | 3.9 | 1.7 | 0.9 | 0.4 | 49.8 | | | | 56.7 |
| Vancomycin | | 3.0 | 50.2 | 46.8 | | | | | | | | | | | 0 |
| Ciprofloxacin | | 15.6 | 76.2 | 5.2 | 0.4 | | 0.4 | 1.7 | 0.4 | | | | | | 2.6 |
| Bacitracin | | | | | | 0.4 | 0.4 | 7.4 | 19.9 | 37.7 | 4.8 | 29.4 | | | 34.2 |
| Flavomycin | | | | | 95.7 | 3.0 | 0.9 | | | | | | 0.4 | | 0.4 |
| Salinomycin | | 2.2 | 48.5 | 16.0 | 21.2 | 10.4 | 1.7 | | | | | | | | 12.1 |
| Quinu/dalfopristin | | 0.9 | | 0.9 | 3.5 | 39.8 | 52.8 | 1.7 | 0.4 | | | | | | 0.4 |
| Genta > 500 | | | | | | | | | | 95.7 | 0.4 | 0.9 | 0.4 | 2.6 | 3.0 |
| Strep > 2000 | | | | | | | | | | | | 58.9 | 1.7 | 39.4 | 39.4 |
| Chloramphenicol | | | | | 6.5 | 73.6 | 4.8 | 1.3 | 12.1 | 1.7 | | | | | 13.9 |
| ***E. faecium*** **(N = 443)** | **MIC (%) distribution (mg/L)** | | | | | | | | | | | | | | |
| | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** | **1024** | **2048** | **R (%)** |
| Amoxicillin | | | 60.5 | 31.2 | 5.0 | 0.2 | | 2.3 | 0.7 | 0.2 | | | | | 3.4 |
| Linezolid | | | 0.5 | 86.9 | 12.4 | 0.2 | | | | | | | | | 0.2 |
| Tetracycline | | 26.2 | 11.7 | 0.2 | | 0.2 | 0.5 | 1.4 | 19.6 | 37.2 | 2.9 | | | | 61.9 |
| Erythromycin | | | 21.7 | 24.2 | 8.1 | 5.0 | 1.8 | 0.5 | 0.2 | 0.2 | 38.4 | | | | 46.0 |
| Vancomycin | | 78.6 | 12.2 | 8.6 | | | | | | 0.7 | | | | | 0.7 |
| Ciprofloxacin | | 11.1 | 23.0 | 28.4 | 30.2 | 5.2 | 1.8 | 0.2 | | | | | | | 7.2 |
| Bacitracin | | | | 0.7 | 8.1 | 3.6 | 1.4 | 1.8 | 9.5 | 28.4 | 20.8 | 25.7 | | | 84.4 |
| Flavomycin | | | | | | | 0.2 | 0.7 | 1.1 | 0.7 | 0.7 | 8.8 | 87.8 | | 99.8 |
| Salinomycin | | 0.5 | 19.0 | 36.6 | 4.1 | 32.1 | 7.9 | | | | | | | | 40.0 |
| Quinu/dalfopristin | | 13.3 | 6.5 | 22.1 | 49.2 | 5.9 | 2.0 | 0.9 | | | | | | | 58.0 |
| Genta > 500 | | | | | | | | | | 99.3 | 0.2 | 0.2 | | 0.2 | 0.2 |
| Strep > 2000 | | | | | | | | | | | | 72.5 | 2.3 | 25.3 | 25.3 |
| Chloramphenicol | | | | | 4.3 | 80.1 | 7.9 | 7.7 | | | | | | | 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 breakpoint.

**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 2005.**

| E. faecalis | Dairy cows (26) | Veal calves (48) | Slaughter pigs (34) | Broiler chickens (123) |
|---|---|---|---|---|
| Amoxicilline | 0 | 0 | 0 | 0 |
| Linezolid | 0 | 0 | 0 | 0 |
| Tetracyline | 23.1 | 85.4 | 76.5 | 78 |
| Erythromycine | 15.4 | 62.5 | 41.2 | 67.5 |
| Vancomycine | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 0 | 8.3 | 0 | 1.6 |
| Bacitracine | 38.5 | 27.1 | 5.9 | 43.9 |
| Flavomycine | 0 | 0 | 2.9 | 0 |
| Salinomycine | 0 | 0 | 5.9 | 21.1 |
| Quinu/dalfopristine | 0 | 2.1 | 0 | 0 |
| Genta > 500 | 3.8 | 10.4 | 8.8 | 0 |
| Strep > 2000 | 26.9 | 66.7 | 38.2 | 31.7 |
| Chlooramfenicol | 7.7 | 43.8 | 5.9 | 5.7 |

| E. faecium | Dairy cows (78) | Veal calves (84_ | Slaughter pigs (128) | Broiler chickens (153) |
|---|---|---|---|---|
| Amoxicilline | 1.3 | 10.7 | 0 | 3.3 |
| Linezolid | 0 | 1.2 | 0 | 0 |
| Tetracycline | 6.4 | 70.2 | 76.6 | 73.2 |
| Erythromycine | 15.4 | 60.7 | 32.8 | 64.7 |
| Vancomycine | 0 | 0 | 0.8 | 1.3 |
| Ciprofloxacin | 10.3 | 8.3 | 3.9 | 7.8 |
| Bacitracine | 97.4 | 94 | 64.1 | 89.5 |
| Flavomycine | 100 | 100 | 100 | 99.3 |
| Salinomycine | 0 | 1.2 | 53.1 | 70.6 |
| Quinu/dalfopristine | 44.9 | 59.5 | 53.9 | 67.3 |
| Genta > 500 | 1.3 | 0 | 0 | 0.7 |
| Strep > 2000 | 7.7 | 54.8 | 9.4 | 31.4 |
| Chlooramfenicol | 0 | 0 | 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. Acquired resistance to the streptogramin combination quinu/dalfopristin (Synercid®) is primarily observed in *E. faecium.* However the MIC R-breakpoint for *E. faecalis* has been increased to 32 mg/L. For *E. faecium* which is intrinsically susceptible at a lower level that *E. faecalis,* the breakpoint is the CLSI advised 2 mg/L. This breakpoint does not adequately differentiate between wild type and resistant subpopulation. Therefore the resistance proportions for *E. faecium* may be an overestimation of the true resistance levels.

**Figure 22. Trends in resistance percentages of *E. faecium* and *E. faecalis* isolated from slaughter pigs, broilers and veal calves in The Netherlands from 1996 - 2005**

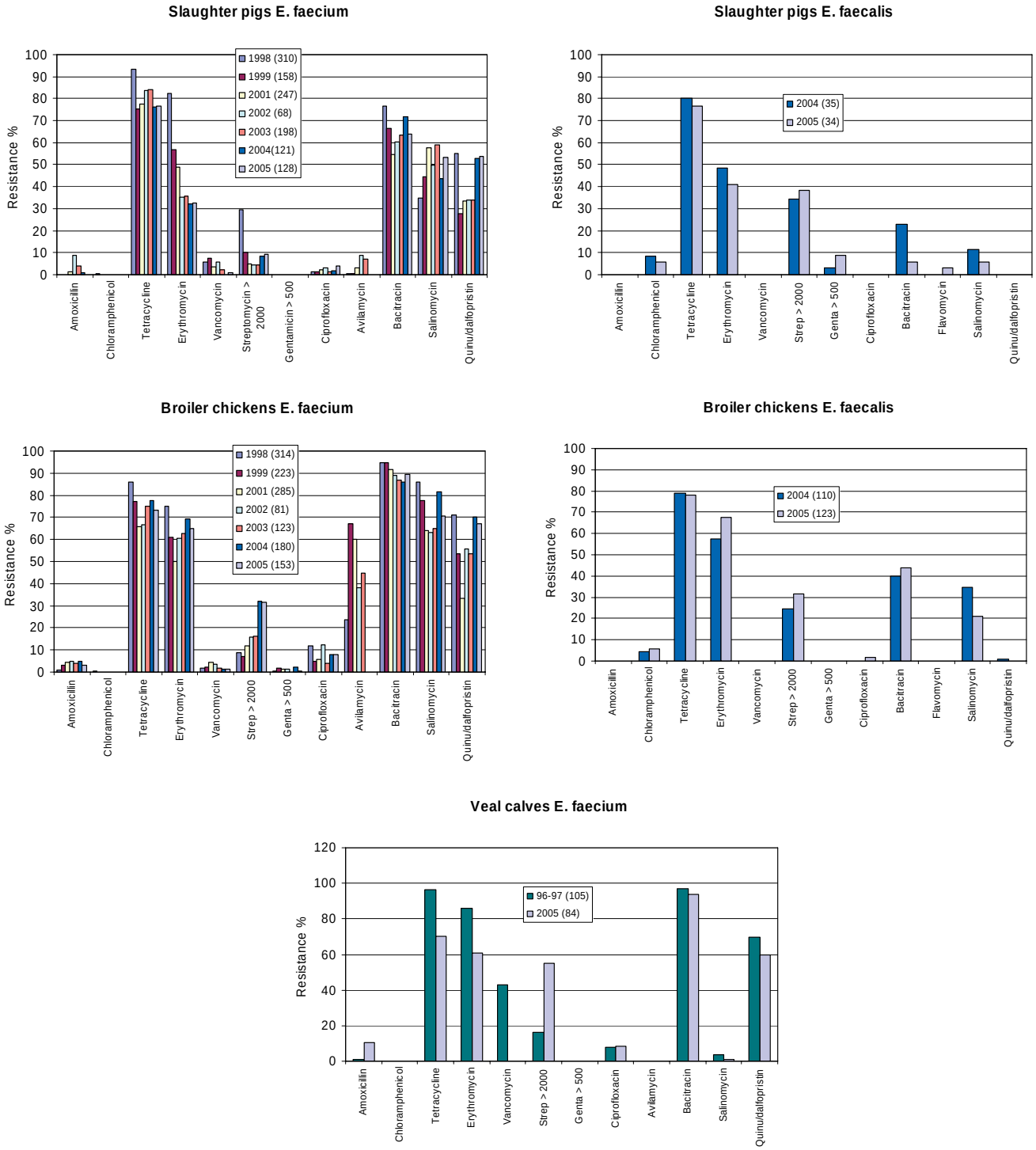
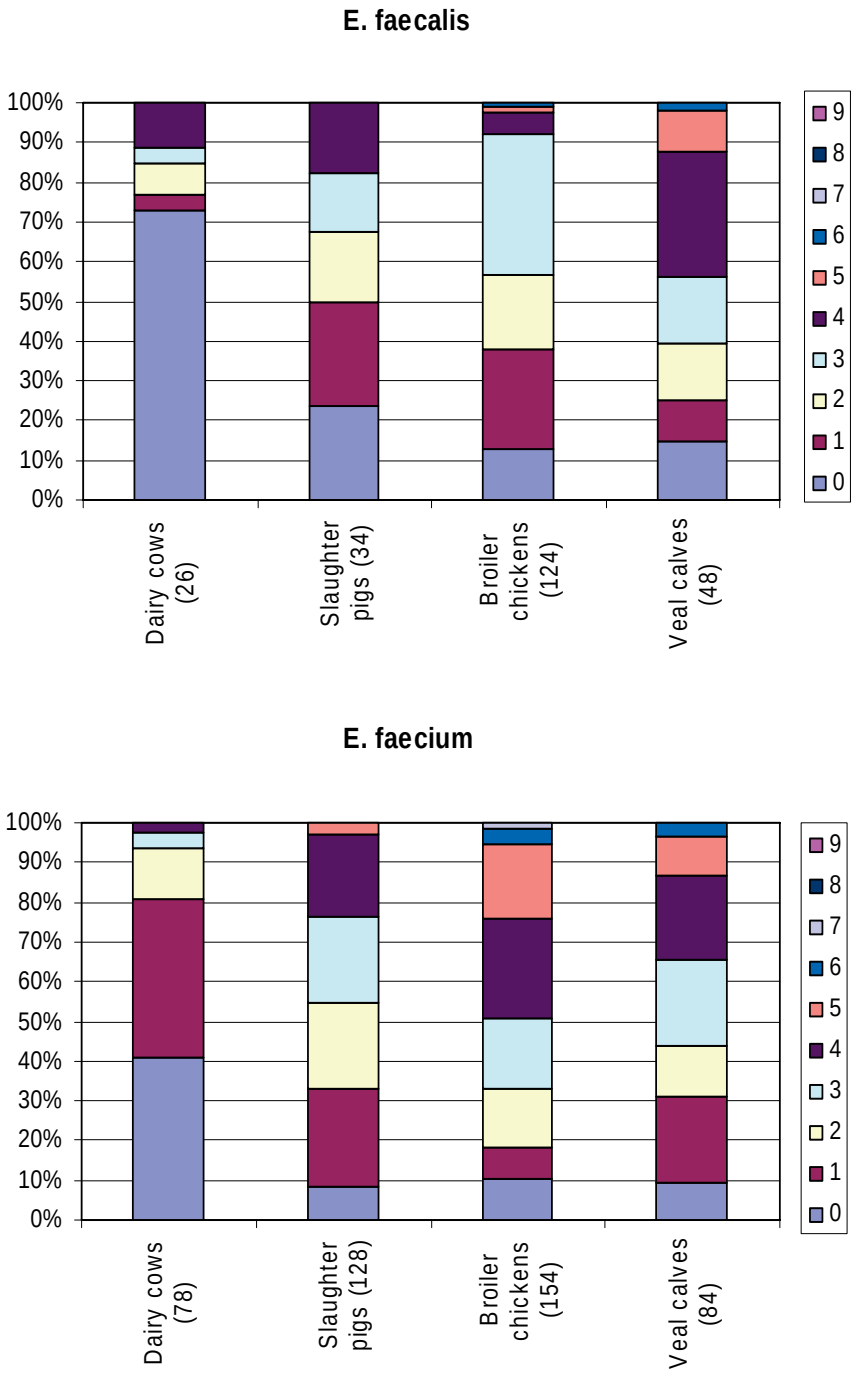



Figure 22 shows the variation in resistance levels over time. A decrease is observed in resistance to vancomycin and erythromycin (pigs only), both former growth promoters which use was banned in the late 1990s. Other drugs show a steady state level of resistance or an increase (high level streptomycin resistance, amoxicillin in veal calves, quinu/dalfopristin).
Multi drug resistance is very common in veal calves, pigs and broilers, but not in dairy cows (Fig. 23).

**Figure 23. Percentages of *E. faecalis* and *E. faecium* strains fully susceptible, resistant to one, two, up to nine classes of antibiotics in dairy cows, slaughter pigs, broiler chickens and veal calves in The Netherlands in 2005.**



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

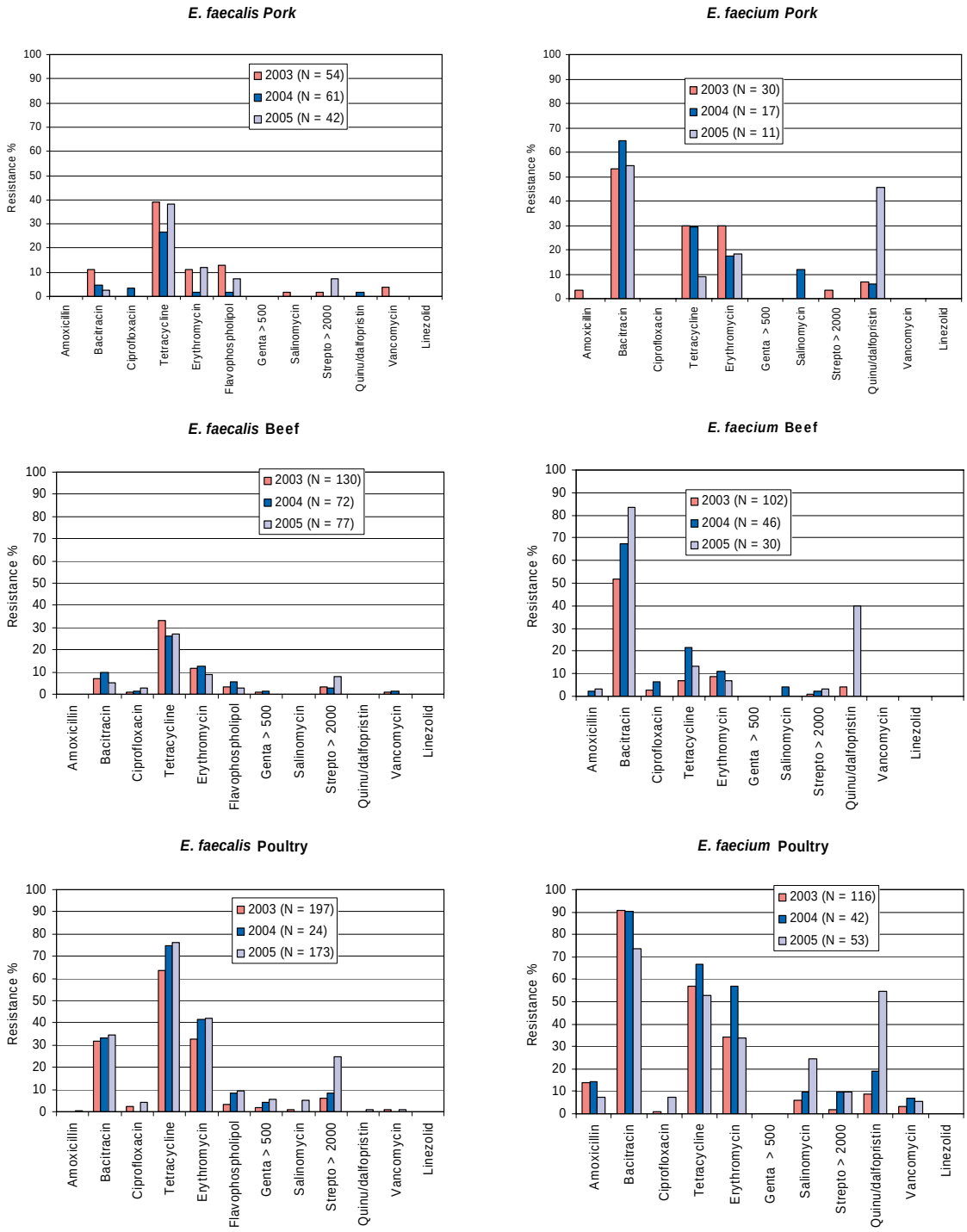
**Table 25. Resistance % of *E. faecalis* and *E. faecium* isolated from raw meat products from poultry, beef, pork, lamb and veal in the Netherlands in 2005**

| | Poultry | Beef | Pork | Lamb | Veal |
|---|---|---|---|---|---|
| ***E. faecalis*** | **N =173** | **N = 77** | **N = 42** | **N = 12** | **N = 25** |
| Amoxicillin | 0.6 | 0 | 0 | 0 | 4 |
| 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 |
| Vancomycin | 1.2 | 0 | 0 | 0 | 8 |
| Ciprofloxacin | 4 | 2.6 | 0 | 0 | 2 |
| Bacitracin | 34.7 | 5.2 | 2.4 | 8.3 | 16 |
| Flavophospholipol | 9.2 | 2.6 | 7.1 | 8.3 | 16 |
| Salinomycin | 5.2 | 0 | 0 | 0 | 0 |
| Quinu/dalfopristin | 1.2 | 0 | 0 | 0 | 0 |
| Genta > 500 | 5.8 | 0 | 0 | 8.3 | 8 |
| Strep > 2000 | 24.9 | 8.1 | 7.1 | 25 | 36 |
| Chloramphenicol | 3.5 | 3.9 | 2.4 | 0 | 24 |
| | **Poultry** | **Beef** | **Pork** | **Lamb** | **Veal** |
| ***E. faecium*** | **N = 53** | **N = 30** | **N = 11** | **N = 4** | **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 | 6.7 | 18.2 | 0 | 11.1 |
| Vancomycin | 5.7 | 0 | 0 | 0 | 0 |
| Ciprofloxacin | 7.5 | 0 | 0 | 0 | 0 |
| Bacitracin | 73.6 | 83.3 | 54.5 | 75 | 55.6 |
| Salinomycin | 24.5 | 0 | 0 | 0 | 0 |
| Quinu/dalfopristin | 54.7 | 40 | 45.5 | 75 | 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 |

In comparison with isolation rates from faecal samples, *E. faecalis* was more frequently isolated than *E. faecium*, which 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 only found in isolates from broilers, poultry products and veal. Also in raw meat products 2 linezolid resistant isolates were detected in poultry products, one with MIC 8 mg/L, the other with MIC 64 mg/L.

Figure 24 shows the trend from 2003 to 2005 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.*

**Figure 24. 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 2005**



# *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.
A resistance trend analysis is very complicated because the population of isolates in the sample can be substantially biased. 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**
Resistance to tetracycline occurred most frequently in both species, but, as was observed for amoxicillin, predominantly in *M. haemolytica* (MHA).
The resistance level of enrofloxacin was somewhat misleading in MHA, 2.5% were classified resistant, but another 20% showed reduced susceptibility, indicating the presence of acquired resistance. Resistance to ceftiofur, tilmicosin and florfenicol was not detected.
Strains isolated from veal calves were without exception more resistant than those from dairy cattle, reflecting the difference in use practices of antibiotics in these animal husbandry systems.

In Table 26 the MIC distributions are presented for both *Pasteurella multocida* (PMU) and *Mannheimia haemolytica* (MHA). In Figure 25 the trends in resistance percentages are presented from 1996 to 2005.

Instead of the previous chapters where for the calculation of resistance percentages, epidemiological cut off values for the wild type distribution were used, for animal pathogens clinical breakpoints were used. The main reason is that epidemiological cut off values have not yet been established for these organisms. Table 26 shows that clinical breakpoints not always optimally distinguish resistant subpopulations (e.g. tetracycline, flumequine and chloramphenicol). However, for most antibiotics they are quite adequate.
The resistance profiles of PMU and MHA were not identical. Tetracycline resistance occurred most frequently in both species, although substantially more frequently in MHA. Also amoxicilline resistance occurs more frequently in MHA. Resistance to aminoglycosides is present in single isolates in both genera.
The resistance percentages for the quinolones are misleading. Based on the R breakpoint $\geq$ 2 mg/L, 5.4% PMU and 2.5% MHA are classified as resistant to enrofloxacin. However, for MHA a substantial population (app. 20%) with reduced susceptibility to enrofloxacin exists (MICs 0.25 – 0.5 mg/L), demonstrating that acquired resistance to quinolones is present in more than 20% of the MHA isolates.
Resistance to ceftiofur, tilmicosin and florfenicol was not detected.

Figure 25 shows that the resistance percentages vary substantially over the years. Trends in resistance cannot be detected.

Table 27 shows the comparison in resistance levels between strains isolates from veal calves and those from dairy cattle. Without exception isolates from veal calves are more resistant. This was expected because in veal calves where mass medication occurs frequently, the selection pressure can be expected to be much higher than in dairy cattle. This is also reflected in the proportions of multidrug resistant MHA and PMU isolates (fig. 26).

**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 2004 and 2005.**

| | MIC % distribution (mg/L) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***P. multocida (37)*** | 0.015 | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | R% |
| Amoxicillin | | | | | 97.3 | | | | | | | | 2.7 | | | 2.7 |
| Ceftiofur | | | 91.9 | 5.4 | 2.7 | | | | | | | | | | | 0 |
| Tetracycline | | | | | 10.8 | 54.1 | 5.4 | 10.8 | | 5.4 | 5.4 | 8.1 | | | | 13.5 |
| Neomycin | | | | | | | | 37.8 | 40.5 | 13.5 | | | | 8.1 | | 8.1 |
| Gentamicin | | | | | | | 43.2 | 35.1 | 16.2 | | | | | 5.4 | | 5.4 |
| Spectinomycin | | | | | | | | | | 8.3 | 47.2 | 38.9 | 2.8 | 2.8 | | 2.8 |
| Trim/sulpha | | | | 75.7 | 10.8 | 5.4 | 2.7 | 2.7 | | | 2.7 | | | | | 2.7 |
| Flumequine | | | | | 83.8 | 5.4 | 2.7 | 2.7 | | | | | 5.4 | | | 5.4 |
| Enrofloxacin | | 91.9 | | 2.7 | | | | | | 5.4 | | | | | | 5.4 |
| Tilmicosin | | | | | | 2.7 | 13.5 | 18.9 | 35.1 | 24.3 | 5.4 | | | | | 0 |
| Chloramphenicol | | | | | | 48.6 | 32.4 | 2.7 | | 2.7 | 13.5 | | | | | 0 |
| Florfenicol | | | | | 2.7 | 83.8 | 13.5 | | | | | | | | | 0 |
| | **MIC (%) distribution (mg/L)** | | | | | | | | | | | | | | | |
| ***M. haemolytica (40)*** | 0.015 | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | R% |
| Amoxicillin | | | | | 85.0 | | | | | | | | 15.0 | | | 15.0 |
| Ceftiofur | | | 100 | | | | | | | | | | | | | 0 |
| Tetracycline | | | | | | 45.0 | 10.0 | | 2.5 | 5.0 | 20.0 | 15.0 | 2.5 | | | 37.5 |
| Neomycin | | | | | | | | 5.0 | 75.0 | 17.5 | | | | 2.5 | | 2.5 |
| Gentamicin | | | | | | 2.5 | 27.5 | 65.0 | 2.5 | | | | | 2.5 | | 2.5 |
| Spectinomycin | | | | | | | | | | | 10.0 | 85.0 | 2.5 | | 2.5 | 2.5 |
| Trim/sulpha | | | | 72.5 | | 12.5 | 5.0 | 7.5 | | | 2.5 | | | | | 2.5 |
| Flumequine | | | | | 57.5 | 15.0 | 2.5 | 2.5 | 20.0 | | | 2.5 | | | | 2.5 |
| Enrofloxacin | | 52.5 | 17.5 | 7.5 | 12.5 | 7.5 | | 2.5 | | | | | | | | 2.5 |
| Tilmicosin | | | | | | | | 5.0 | 52.5 | 27.5 | 15.0 | | | | | 0 |
| Chloramphenicol | | | | | | 5.0 | 35.0 | 35.0 | | 2.5 | 2.5 | 15.0 | 5.0 | | | 20.0 |
| Florfenicol | | | | | | 10.0 | 72.5 | 15.0 | 2.5 | | | | | | | 0 |

**Figure 25. Trends in resistance (in %) of *P. multocida* and *M. haemolytica* isolated from 1996 – 2005 in the Netherlands.**

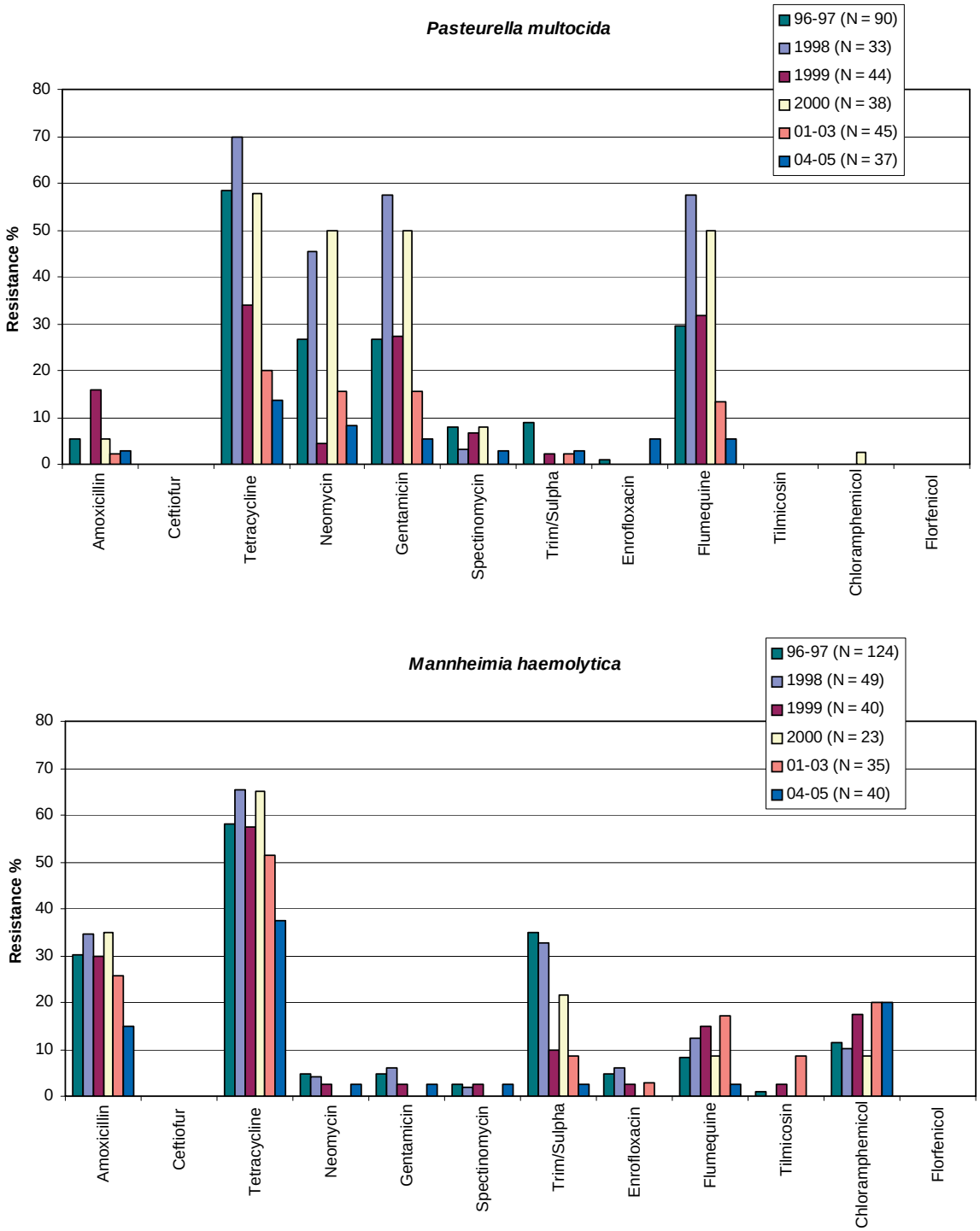
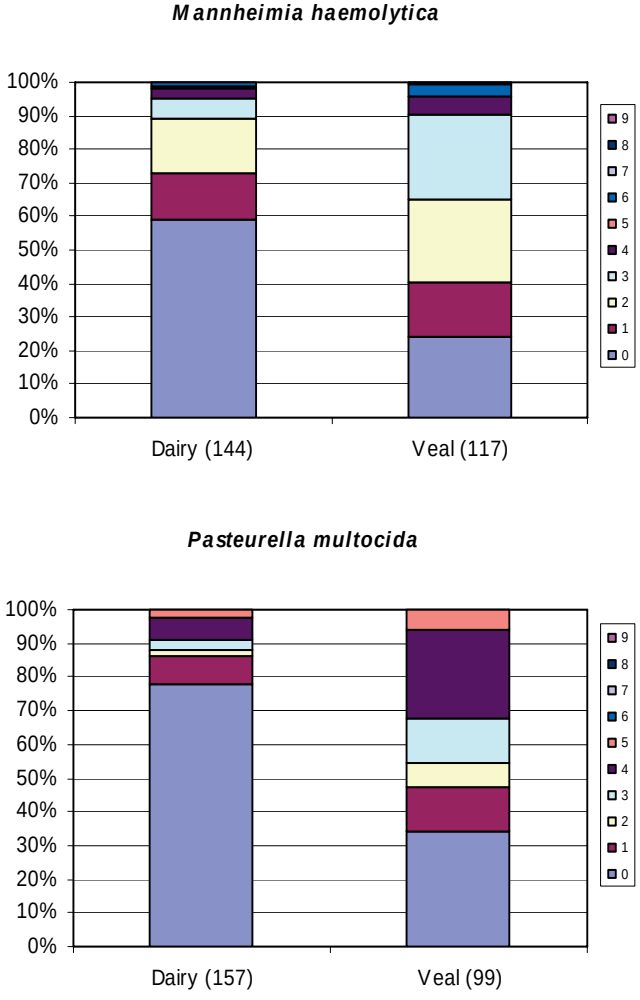


**Table 27. Resistance percentages of *M. haemolytica* (MHA) and *P. multocida* (PMU) isolated from veal calves and dairy cattle in the Netherlands from 1996 – 2005.**

| | Veal calves | | Dairy cattle | |
|---|---|---|---|---|
| **1996 - 2005** | **MHA** | **PMU** | **MHA** | **PMU** |
| **N** | **117** | **99** | **145** | **156** |
| Amoxicillin | 41.0 | 12.2 | 17.2 | 1.9 |
| Ceftiofur | 0 | 0 | 0 | 0 |
| Tetracycline | 74.6 | 48.0 | 37.2 | 17.3 |
| Neomycin | 4.3 | 36.4 | 2.1 | 12.2 |
| Gentamicin | 5.1 | 45.5 | 1.4 | 12.2 |
| Spectinomycin | 2.6 | 7.1 | 0.7 | 2.6 |
| Trim/sulphamethoxazole | 31.6 | 2.0 | 10.3 | 0.6 |
| Enrofloxacin | 12.0 | 8.8 | 2.8 | 1.3 |
| Flumequine | 13.7 | 48.5 | 8.3 | 12.7 |
| Tilmicosin | 0 | 0 | 0.7 | 0 |
| Chloramphenicol | 16.5 | 1.1 | 10.4 | 0 |
| Florfenicol | 0 | 0 | 0 | 0 |

**Figure 26. Percentages of *M. haemolytica* and *P. multocida* strains fully susceptible, resistant to one to a maximum of nine antimicrobial classes in dairy cattle and veal calves in the Netherlands**



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

**Highlights**

In general *E. coli* strains isolated from milk samples from cows suffering from mastitis were susceptible to most antibiotic classes. Only resistance to the conservative antibiotics (amoxicillin, tetracycline, streptomycin and trim/sulpha) occurred more frequently than in single isolates.
The coliform bacteria showed a high level of resistance to amoxicillin and to the combination with clavulanic acid and cefuroxime. All isolates were susceptible to cefoperazone and cefquinome.
The *S. aureus* isolates tested were susceptible to most antibiotics. 7.1% were penicillin resistant. Oxacillin resistance (MRSA) was not present. The coagulase negative staphylococci were more resistant than *S. aureus.* 61.1% were resistant to penicillin and 5.2% to oxacillin (*mecA*-positive).
Based in the CLSI criteria in the streptococci only resistance to erythromycin, lincomycin, pirlimycin and tetracycline was observed. However, more than 30% of *S. uberis* showed reduced susceptibility to penicillin. In 2005 *S. uberis* was more frequently resistant to erythromycin, lincomycin and pirlimycin than *S. dysgalactiae*. Resistance to tetracycline was highest in *S. dysgalactiae*.

**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 2005.**

| | MIC (%) distribution (mg/L) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **E. coli (N = 84)** | **0.03** | **0.06** | **0.125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **R%** |
| Amoxicillin | | | | | | 2.4 | 36.9 | 36.9 | 14.3 | | | 9.5 | | 9.5 |
| Amox-clavulanic acid | | | | | | 1.2 | 50.0 | 36.9 | 10.7 | 1.2 | | | | 0 |
| Cefquinome | | 92.9 | 6.0 | | | 1.2 | | | | | | | | 0 |
| Cefoperazone | | | 36.9 | 47.6 | 4.8 | 3.6 | 6.0 | 1.2 | | | | | | 0 |
| Cefuroxime | | | | | | | 19.0 | 63.1 | 17.9 | | | | | 0 |
| Tetracycline | | | | | 1.2 | 46.4 | 39.3 | 4.8 | | | | 8.3 | | 8.3 |
| Gentamicin | | | | | 56.0 | 36.9 | 6.0 | 1.2 | | | | | | 0 |
| Kanamycin | | | | | | | 14.3 | 76.2 | 7.1 | 1.2 | 1.2 | | | 1.2 |
| Neomycin | | | | | | 63.1 | 34.5 | 1.2 | | | 1.2 | | | 1.2 |
| Streptomycin | | | | | | | | 2.4 | 77.4 | 9.5 | 2.4 | 1.2 | 7.1 | 8.3 |
| Enrofloxacin | 88.1 | 10.7 | | | 1.2 | | | | | | | | | 0 |
| Trim/Sulpha | | | 91.7 | 1.2 | 1.2 | | | | | | 6.0 | | | 6.0 |
| | **MIC (%) distribution (mg/L)** | | | | | | | | | | | | | |
| **Coliform (N = 85)** | **0.03** | **0.06** | **0.125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **R%** |
| Amoxicillin | | | | | | 2.4 | 4.7 | 4.7 | | | 7.1 | 81.2 | | 88.3 |
| Amox-clavulanic acid | | | | | 1.2 | 3.5 | 50.6 | 9.4 | 5.9 | 1.2 | 9.4 | 14.1 | 4.7 | 28.2 |
| Cefquinome | | 60.0 | 30.6 | 5.9 | 2.4 | | 1.2 | | | | | | | 0 |
| Cefoperazone | | 3.5 | 14.1 | 25.9 | 22.4 | 7.1 | 8.2 | 9.4 | 3.5 | 5.9 | | | | 0 |
| Cefuroxime | | | | | | 2.4 | 32.9 | 24.7 | 11.8 | 15.3 | 12.9 | | | 12.9 |
| Tetracycline | | | | | 5.9 | 16.5 | 41.2 | 14.1 | | | 7.1 | 15.3 | | 22.4 |
| Gentamicin | | | | 18.8 | 62.4 | 16.5 | 2.4 | | | | | | | 0 |
| Kanamycin | | | | | | 3.5 | 64.7 | 17.6 | 3.5 | 1.2 | 9.4 | | | 9.4 |
| Neomycin | | | | | 23.5 | 63.5 | 5.9 | 1.2 | | | 5.9 | | | 5.9 |
| Streptomycin | | | | | | | 4.7 | 55.3 | 17.6 | 3.5 | 3.5 | 5.9 | 9.4 | 15.3 |
| Enrofloxacin | 52.9 | 31.8 | 12.9 | | 2.4 | | | | | | | | | 0 |
| Trim/Sulpha | | | 81.2 | 7.1 | 3.5 | | | | | | 8.2 | | | 8.2 |

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.

*E. coli* strains isolated from milk samples from cows suffering from mastitis were in general susceptible to the antibiotics included in the panel. Only resistance to the conservative antibiotic classes amoxicillin. streptomycin. trim/sulpha and tetracycline was present in significant percentages. All strains were susceptible to amoxicilline-clavulanic acid, the 2nd (cefuroxime) and 3rd generation cefalosporin (cefoperazone) and the cefamycin (cefquinome) tested. One isolate showed reduced susceptibility to cefquinome (MIC 1 mg/L). Because reduced susceptibility to cefalosporins indicates the presence of ESBLs, this isolate was further tested for susceptibility to cefotaxime, ceftazidime, the combination of these antibiotics with clavulanic acid and cefoxitin. These results of these tests showed that this isolate was susceptible to all these drugs and the isolate was not ESBL positive. Resistance to the aminoglycosides occurred occasionally and resistance to enrofloxacin was absent.

The coliform bacteria (27.3% *Enterobacter* spp.. 59% *Klebsiella* spp.. *9.4% Citrobacter* spp. and 9.4% *Serratia* spp.) showed a high level of resistance to amoxicillin and to amoxicilline-clavulanic acid. All isolates were susceptible to the third generation cephalosporin, cefoperazone and cefquinome, gentamicin and the fluoroquinolon, enrofloxacin.

Fig. 25 demonstrates that from 2002 to 2005 the resistance levels for *E. coli* show a tendency to decrease, whilst those for the coliform bacteria show a tendency to increase.

**Figure 27. Trends in resistance percentages for *E. coli* and coliform bacteria isolated from clinical mastitis cases in dairy cattle in the Netherlands from 2002 – 2005.**

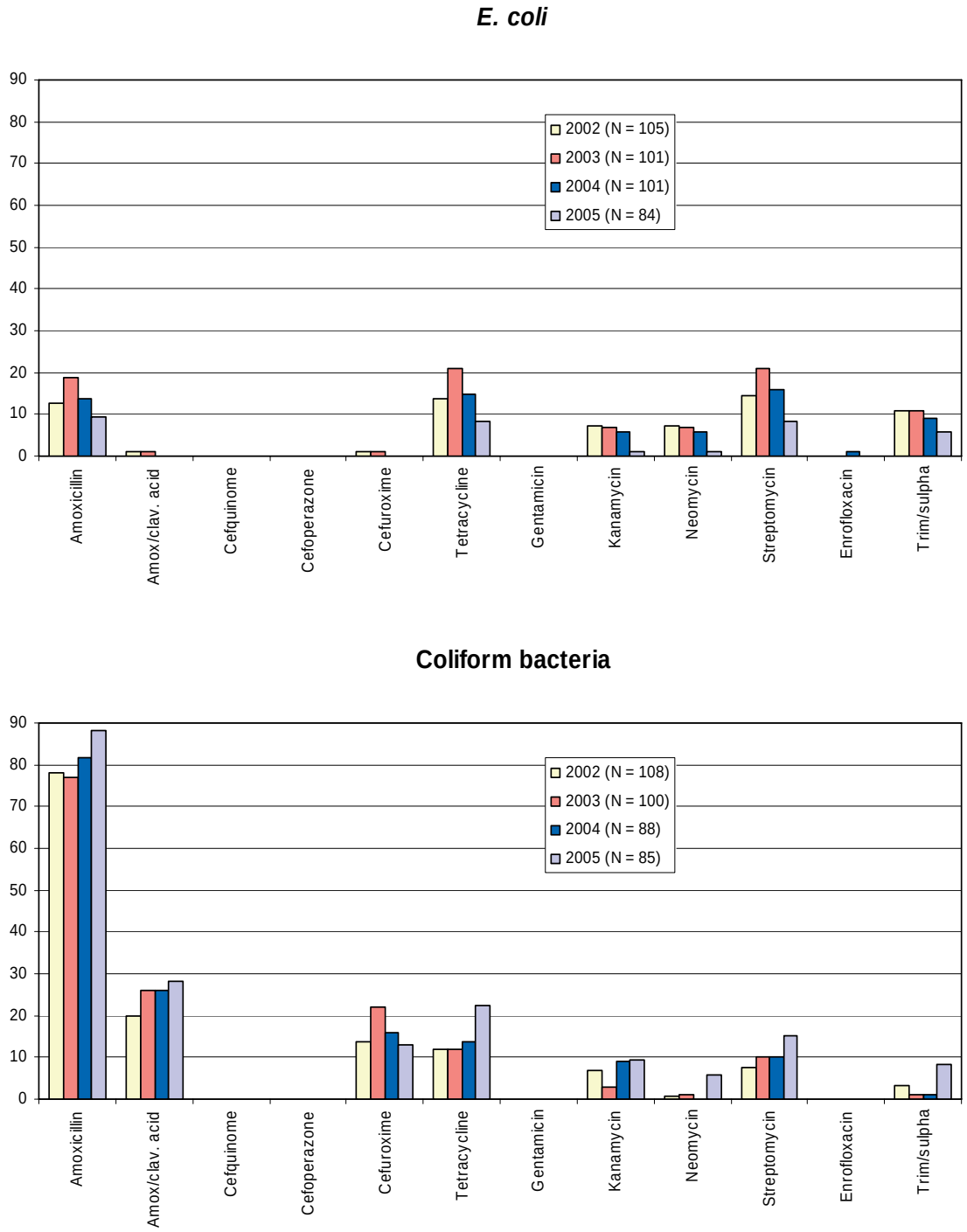


**Table 29. MIC-distributions (in %) of *S. aureus* and coagulase-negative staphylococci (CNS) isolated from clinical mastitis cases in dairy cattle by the Animal Health Service in Deventer in 2005.**

| | MIC (%) distribution (mg/L) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. areus* (N = 99) | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | R% |
| Penicillin | 86.9 | 6.1 | | | 1.0 | 2.0 | 2.0 | 2.0 | | | | | 7.1 |
| Oxacillin | | 3.0 | 26.3 | 59.6 | 11.1 | | | | | | | | 0 |
| Amox-clavulanic acid | 50.5 | 31.3 | 14.1 | 4.0 | | | | | | | | | 0 |
| Cephalothin | 1.0 | 29.3 | 44.4 | 25.3 | | | | | | | | | 0 |
| Tetracycline | | | 2.0 | 94.9 | 3.0 | | | | | | | | 0 |
| Kanamycin | | | | | 2.0 | 35.4 | 54.5 | 7.1 | 1.0 | | | | 0 |
| Neomycin | | 1.0 | 10.1 | 57.6 | 29.3 | 2.0 | | | | | | | 0 |
| Streptomycin | | | | | | | 6.3 | 64.6 | 27.1 | 2.1 | | | 2.1 |
| Erythromycin | | | 7.1 | 91.9 | 1.0 | | | | | | | | 0 |
| Clindamycin | | | 2.0 | | 78.8 | 15.2 | | | 1.0 | 1.0 | 2.0 | | 4.0 |
| Pirlimycin | | | 3.0 | 66.7 | 27.3 | 1.0 | 2.0 | | | | | | 2.0 |
| Trim/sulpha | | 100.0 | | | | | | | | | | | 0 |
| | MIC (%) distribution (mg/L) | | | | | | | | | | | | |
| CNS (N = 95) | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | R% |
| Penicillin | 59.2 | 3.2 | 13.8 | 3.2 | 9.6 | 4.3 | 1.1 | 1.1 | 4.3 | | | | 61,1# |
| Oxacillin | 9.5 | 30.5 | 36.8 | 10.5 | 5.3 | 1.1 | 3.2 | | 3.2 | | | | 5.2* |
| Amox-clavulanic acid | | 56.8 | 28.4 | 10.5 | 3.2 | 1.1 | | | | | | | 5.2* |
| Cephalothin | 4.2 | 24.2 | 53.7 | 5.3 | 9.5 | 1.1 | 1.1 | 1.1 | | | | | 5.2* |
| Tetracycline | | 10.5 | 62.1 | 13.7 | 1.1 | 3.2 | | | | 9.5 | | | 9.5 |
| Kanamycin | | | | 10.5 | 60.0 | 16.8 | 9.5 | 2.1 | | | | 1.1 | 1.1 |
| Neomycin | | 63.2 | 22.1 | 8.4 | 4.2 | 1.1 | | 1.1 | | | | | 0 |
| Streptomycin | | | | | 8.4 | 30.5 | 37.9 | 12.6 | 2.1 | 1.1 | 4.2 | 3.2 | 8.5 |
| Erythromycin | | 11.7 | 56.4 | 23.4 | 1.1 | 1.1 | 2.1 | 1.1 | | 3.2 | | | 4.3 |
| Clindamycin | 11.6 | 41.1 | 26.3 | 10.5 | 6.3 | 1.1 | 1.1 | | 2.1 | | | | 3.2 |
| Pirlimycin | | 3.2 | 31.6 | 42.1 | 8.4 | 6.3 | 4.2 | 2.1 | | 2.1 | | | 8.4 |
| Trim/sulpha | | 77.9 | 11.6 | 8.4 | 1.1 | 1.1 | | | | | | | 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. The vertical bars indicate the breakpoints.
# 25 isolates with penicillin MIC ≤ 0.06 mg/L were positive for penicillinase production. Therefore the accurate penicillin resistance percentage was 61.1% in stead of 37.4%.
* All but one isolate with MIC ≥ 4 µg/ml (5.2%) 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 2005 7.1% of the isolates were penicillinase producers but oxacillin resistance was not detected. 4% were resistant to clindamycin and 2% to the related but more potent lincosamide drug pirlimycin.

The coagulase negative staphylococci were more resistant than *S. aureus.* In 2005, 61.1% were classified resistant to penicillin. This seems to be a substantial increase compared to previous years (40.8% reported in 2004), but this is not the case. Penicillin resistance in CNS is partly caused by penicillinase production encoded by the *bla*$_Z$-gene. This gene is inducible in staphylococci. In broth microdilution induction is not optimal, therefore in 2005 (and retrospectively also for 2004) all isolates that were initially classified S, were tested for penicillinase production by the cloverleaf test. This test resulted in 2005 in 25 additional isolates that were classified penicillin resistant and in 2004 12 additional isolates. This needs to be taken into account in the trends analysis in fig. 28.
oxacilline MICs varied from 2 - > 8 µg/ml.

Resistance to tetracycline (16.3%). lincomycin (14.3%) and streptomycin (11.3%) was quite commonly present. Resistance to pirlimycin was substantially lower (5.1%).

Although the numbers of strains included were relative large, the trends in resistance in fig. 26 may be affected by selection bias and not reflect true trends.

**Figure 28. Trends in resistance percentages for *S. aureus* and coagulase negative staphylococci isolated from mastitis milk in the Netherlands from 2002 - 2005.**

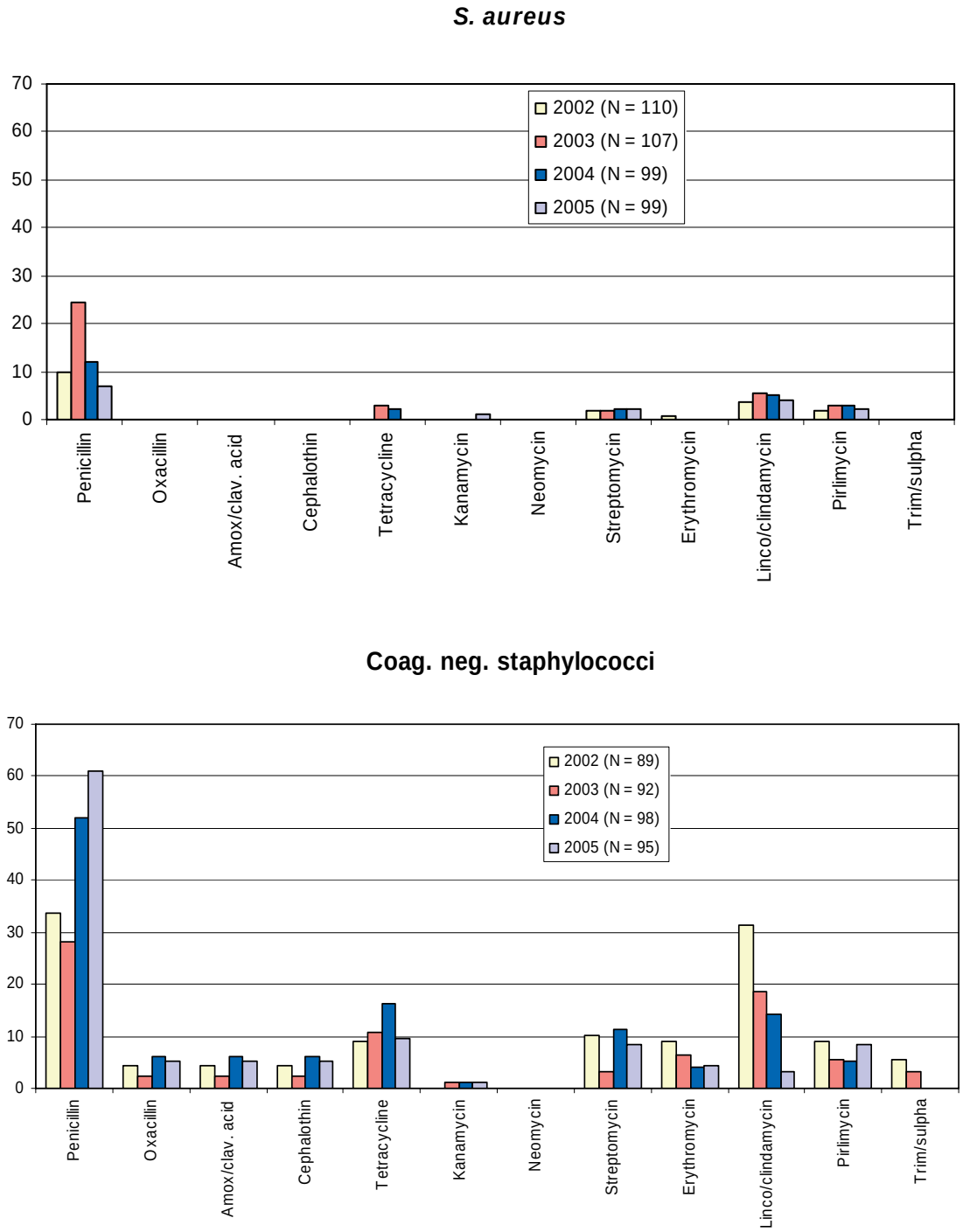


**Table 30. MIC-distributions (in %) of *S. uberis* and *S. dysgalactiae* isolated from mastitis milk samples from Dutch cattle by the Animal Health Service in 2005.**

| *S. uberis* (N = 95) | MIC % distribution (µg/ml) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.015 | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | R% |
| Penicillin | 51.6 | 7.4 | 5.3 | 12.6 | 21.1 | 2.1 | | | | | | | | | | 0.0 |
| Amox/clav. Acid | 1.1 | 47.4 | 12.6 | 9.5 | 29.5 | | | | | | | | | | | 0.0 |
| Cephalothin | | | | 16.8 | 41.1 | 8.4 | 26.3 | 7.4 | | | | | | | | 0.0 |
| Erythromycin | | 4.2 | 75.8 | 5.3 | 1.1 | 1.1 | 4.2 | 2.1 | | | | | 6.3 | | | 12.6 |
| Lincomycin | | | | 28.4 | 3.2 | | 1.1 | 22.1 | 10.5 | 4.2 | | | 30.5 | | | 34.7 |
| Pirlimycin | | 6.3 | 54.7 | 2.1 | 4.2 | 1.1 | | 13.7 | 12.6 | 1.1 | | 1.1 | 3.2 | | | 18.0 |
| Trim/sulpha | | | 3.2 | 43.2 | 51.6 | 2.1 | | | | | | | | | | 0.0 |
| Tetracycline | | | | | 22.1 | 45.3 | 1.1 | | | | | 2.1 | 16.8 | 6.3 | 6.3 | 31.6 |

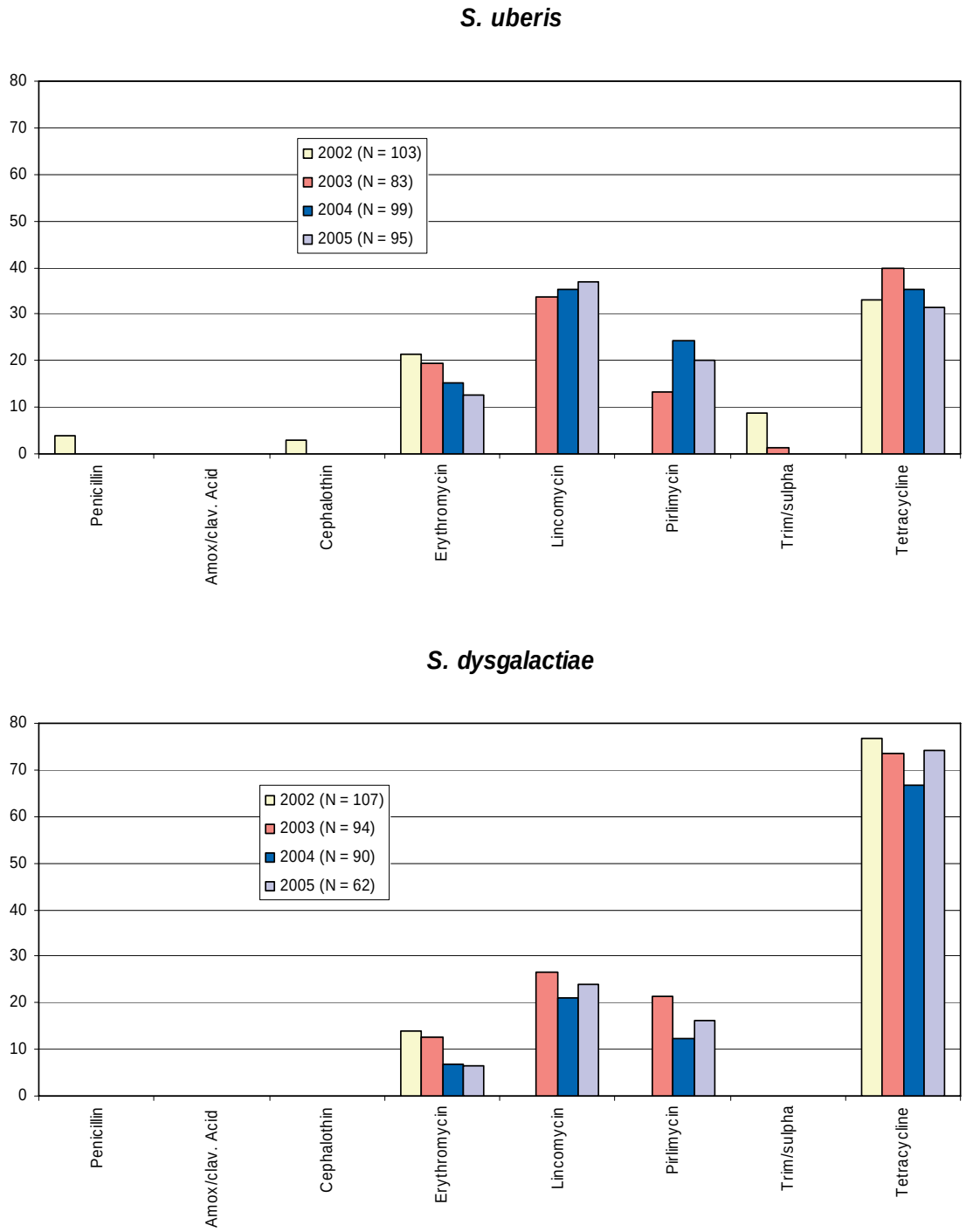
| *S. dysgalactiae* (N = 62) | MIC % distribution (µg/ml) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.015 | 0.03 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | R% |
| Penicillin | 100.0 | | | | | | | | | | | | | | | 0.0 |
| Amox/clav. Acid | 100.0 | | | | | | | | | | | | | | | 0.0 |
| Cephalothin | | | | 85.5 | 14.5 | | | | | | | | | | | 0.0 |
| Erythromycin | | 6.5 | 85.5 | 1.6 | | | | | | | | | 6.5 | | | 6.5 |
| Lincomycin | | | | 16.1 | 37.1 | | 1.6 | 4.8 | 21.0 | 6.5 | 1.6 | | 11.3 | | | 19.4 |
| Pirlimycin | | 11.3 | 67.7 | 4.8 | 4.8 | | | | 4.8 | 3.2 | 1.6 | | 1.6 | | | 11.2 |
| Trim/sulpha | | 1.6 | 66.1 | 32.3 | | | | | | | | | | | | 0.0 |
| Tetracycline | | | | | | | | 1.6 | 6.5 | 17.7 | | | 48.4 | 25.8 | | 74.2 |

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 2005 all isolates of *S. uberis* and *S. dysgalactiae* were susceptible to penicillin and therefore also to all other beta-lactams (Table 30). However *S. uberis* show a bimodal distribution indicating that a 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 frequently in both species. Resistance to tetracycline was very common.

Resistance to erythromycin shows a tendency to decrease since 2002 in both species. Trim/sulpha resistance could not any more be detected in *S. uberis* since 2003. Resistance to the other antibiotics was stable.

**Figure 29. Trends in resistance percentages for *S. uberis* and *S. dysgalactiae* isolated from mastitis milk in The Netherlands from 2002 - 2005.**



# III Appendices

## Appendix I. Methicillin Resistant *S. aureus* (MRSA) in Dutch Food Animals

In 2005 in the Netherlands MRSA was isolated from patients who had contact with pigs.(Voss, Loeffen et al. 2005) Subsequently a point prevalence study in pig farmers was conducted, which revealed that 23% were carrying MRSA, compared to 0.03% carriers in the community. MRSA was again isolated from family members at a pig farm and at that farm 8 out of 10 pigs sampled were found positive for MRSA in their noses.(Huijsdens, van Dijke et al. 2006) All these MRSAs were non-typable (NT) by pulsed field gel electrophoresis (PFGE) using the *Sma*I restriction enzyme. Bens et al. (2006) described that the presence of a restriction modification enzyme, which methylates the *Sma*I-restriction sequence in NT-MRSA is the reason for the fact that PFGE with *Sma*I will not produce a band pattern.
In November 2005, the Institute for Public Health and the Environment (RIVM) in collaboration with the Dutch Consumer Product Safety Authority (VWA) conducted a prevalence study in slaughter pigs by sampling 540 pigs from 54 different slaughter batches at 9 slaughterhouses.(de Neeling, van den Broek et al. 2007) They found 209 pigs (39%) positive for NT-MRSA in their noses and 81% of all farms positive. Molecular typing revealed that all isolates were non-typable by *Sma*I-PFGE and *spa*-typing showed closely related *spa*-types (mainly t011, t108 and t1254). Multi Locus Sequence typing (MLST) revealed that all isolates tested were ST398, a sequence type that was previously only twice recorded in the MLST database on human MRSA-isolates. Three SCC*mec* types were found: III (3%), IVa (39%) and V (57%) and all isolates were PVL-negative. All isolates were *mecA*- positive and also resistant to tetracycline (100%), erythromycin (23%), clindamycin (23%), kanamycin/gentamicin, tobramycin (36%). No ciprofloxacin resistance was detected by de Neeling et al.

Indications on presence of NT-MRSA in other animal species in the Netherlands were found by examining faecal samples from food animals at CIDC-Lelystad. Faecal samples from dairy cows were positive in 1 out of 23 samples, and pig faeces was positive in 1 out of 25 samples. Moreover recently, poultry shedding NT-MRSA and infected poultry farmers were detected, indicating a more widespread occurrence of NT-MRSA in the Netherlands than in pigs only. Moreover in 2005/2006 NT-MRSA was detected by the Animal Health Service in Deventer on three dairy farms in the Netherlands causing clinical mastitis.
A recent publication by Witte et al. (2007) showed the occurrence of MRSA ST398 in humans, a dog, horses and a pigs in Germany and two horses in Austria.(Witte, Strommenger et al. 2007) In Denmark ST398 was detected in human patients in autumn 2006 and in a single swine.(Aarestrup 2007) These data and the fact that large numbers of live pigs and other animals are transported in Europe suggest that NT-MRSA is likely to be present in many countries in Europe.

This unexpected and widespread occurrence of MRSA in food animals was considered a very worrisome situation in the Netherlands, which lead to a coordinated action by the Ministry of Agriculture Nature and Food Quality. First the research priorities were defined (Mevius and Verbrugh 2006), subsequently a research programme coordinated by RIVM and conducted by a collaboration between, RIVM, Utrecht University, VWA, CIDC, the Animal Health Service and Academic Medical Centres was started to act on these priorities. The Faculty of Veterinary Medicine of Utrecht University is the project leader.
This research programme includes activities, which are related to all other projects: harmonisation of detection methods, detection of resistance, genetic characterisation of ST398, and molecular typing of the MRSA strains isolated.
In addition to these generic projects, studies on the prevalence in different animal species and parts of the animal husbandry systems including analysis of risk factors for the occurrence and high or low prevalence of MRSA, transmission of MRSA in animal husbandry systems and slaughterhouses,

transmission of MRSA on farms to humans and MRSA in meat products. Research projects aimed at the human health aspects of this MRSA clone have been initiated as well, they include studies aimed at human to human transmission of NT-MRSA, virulence of NT-MRSA in comparison to MSSA, NT-MRSA carriers in human health care and infection prevention measures for people at risk.

In Dutch hospitals, based on the new information the infection prevention strategy for MRSA has been adjusted. All pig farmers, veterinarians and slaughterhouse personnel when admitted to a hospital will be nursed in isolation until being proven free of MRSA. People related to veal calves will be examined for MRSA but isolated only if MRSA is detected.

**References**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3. Huijsdens, X. W., B. J. van Dijke, et al. (2006). "Community-acquired MRSA and pig-farming." Ann Clin Microbiol Antimicrob 5: 26.
4. Mevius, D. and H. Verbrugh (2006). "Research Priorities for MRSA in Dutch Animal Husbandry (in Dutch)." Tijdschr Diergeneeskd 131(24): 930-3.
5. Voss, A., F. Loeffen, et al. (2005). "Methicillin-resistant Staphylococcus aureus in pig farming." Emerg Infect Dis 11(12): 1965-6.
6. Witte, W., B. Strommenger, et al. (2007). "Methicillin-Resistant *Staphylococcus aureus* in Humans and Animals in Central Europe." Emerging Infectious Diseases 13(2): 255 - 258.

# Appendix II. Materials and Methods

***Salmonella***

A total of 12.567 isolates were tested for antimicrobial susceptibility between 1999-2005 (table 31). Human isolates (N=6794) 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=874) and cattle, including calves (N=355) were sent to the RIVM by the Animal Health Service concerning approximately 80% clinical Salmonella infections. Those from chickens (broilers, including poultry products, N=954; layers, reproduction animals and eggs, N=749) 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. In all years 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 – 2005 in the Netherlands.**

| | Total | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 |
|---|---|---|---|---|---|---|---|---|
| Human | 6794 | 674 | 349 | 1056 | 862 | 1338 | 1339 | 1176 |
| Pig | 874 | 31 | 195 | 114 | 168 | 127 | 119 | 120 |
| Cattle | 355 | 18 | 28 | 56 | 33 | 24 | 106 | 90 |
| Chicken (misc.) | 547 | 0 | 20 | 154 | 142 | 172 | 29 | 30 |
| Broilers (faeces/meat) | 954 | 68 | 100 | 164 | 238 | 192 | 110 | 82 |
| Layers/Repro/Eggs | 749 | 93 | 86 | 80 | 69 | 91 | 93 | 237 |
| Other sources | 2294 | 22 | 22 | 331 | 353 | 486 | 482 | 598 |
| Total | 12567 | 906 | 800 | 1955 | 1865 | 2430 | 2278 | 2333 |

**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. However, if changes span more than one year, the confidence that a change occurred is more favourable compared to what has been tabulated in Table 32.

**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 farms in relation to the zoonoses monitoring programme of the Netherlands. This was coordinated and conducted by the Food and Consumer Product Safety Authority (VWA). The samples were taken throughout the year 2005. At regional laboratories of the VWA and at the Department of Bacteriology and TSEs the microbiological analysis was performed. At CIDC-Lelystad 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 CIDC-Lelystad. For *E. coli* MacConkey agar and for the enterococci Slanetz and Bartley agar was inoculated with 50 µl of serial dilutions of the sample in saline with a spiral plater (enterococci) or direct inoculation of the plates with cotton swabs (*E. coli*). 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 *Campylobacter* 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. 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 CIDC-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 NCCLS guidelines (M31-A2 and M7-A6). For broth micro dilution, microtitre trays were used with dehydrated dilution ranges of custom made panels of antibiotics. Trek Diagnostic Systems, in the UK, manufactured these microtitre trays. For the *Campylobacter* spp., after

inoculation of the microtitre trays with 50 µl of a 200 fold diluted 0.5 McFarland suspensions in saline solution, the trays were incubated micro aerobically in a shaking incubator at 37°C for 48 hours. 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. These were based on MIC-breakpoints listed in tables 33 and 34.

In 2005 for the first year as interpretive criteria not clinical breakpoints prescribed by CLSI were used for *Salmonella, Campylobacter* spp., *E. coli* (as indicator organisms) and enterococci but epidemiological cut-off values for the wild type distribution as defined by representatives from veterinary reference institutes: DFVF Denmark, SVA Sweden, AFSSA France, VLA UK and CIDC Netherlands in collaboration with EUCAST (Table 33). The wild type distributions and the cut-off values can be found at the website www.eucast.org. The reason for changing to epidemiological cut-off values as criteria was to improve the sensitivity of the programme for detection of acquired resistance. This concept is adopted by EFSA as an approach for resistance reporting in the annual Zoonoses Report, which will be implemented in 2007. The EFSA report with the proposal for a harmonized European monitoring scheme[3] can be downloaded from the EFSA homepage (www.efsa.europa.eu) .

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.

For the animal pathogens clinical breakpoints were used (CLSI M31-A2, M100-S17) (Table 34).

[3] Report of 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.

**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 | - | - | - | - |
| Imipenem | 1 | 1 | - | - | - | - |
| Streptomycin | - | 16 | 2 | 4 | 2000 | 2000 |
| Gentamicin | 2 | 2 | 1 | 2 | 500 | 500 |
| Neomycin | 4 | 8 | 1 | 2 | - | - |
| Tetracycline | 8 | 8 | 2 | 2 | 2 | 2 |
| Doxycycline | 8 | 4 | 0.5 | 0.5 | 0.5 | 0.5 |
| Sulphamethoxazole | 256* | 256* | 256 | 32 | - | - |
| Trimethoprim | 2 | 2 | - | - | - | - |
| Trim/sulphamethoxazole | 2/38 | 2/38 | 16/304 | 2/38 | - | - |
| 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 |
| Bacitracin | - | - | - | - | 128 | 128 |
| Flavomycin | - | - | - | - | - | 16 |
| Quinu/dalfopristin | - | - | - | - | 2* | 32 |
| Virginiamycin | - | - | - | - | 4 | 32 |
| Erythromycin | - | - | 4 | 16 | 4 | 4 |
| Linezolid | - | - | - | - | 4* | 4* |
| 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 | * | - |
| Amoxicillin | 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 | - | - | - | 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 |
| Lincomycin | - | - | - | - | - | 4 |
| Clindamycin | - | - | - | 2 | 2 | - |

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


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