MARAN 2004

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
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Colophon

This report is published under the acronym *MARAN-2004* by VANTURES, the Veterinary Antibiotic Usage and Resistance Surveillance Working Group. The information presented in *MARAN-2004* 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.
MARAN-2004 can be ordered from the secretariat of the CIDC-Lelystad, p/a Houtribweg 39, 8221 RA Lelystad, The Netherlands. *MARAN-2004* is also available at the website of CIDC-Lelystad: www.cidc-lelystad.nl.
The citation is: *MARAN-2004* - Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in The Netherlands In 2004

Editors:
Dr. D.J. Mevius
Central Institute for Animal Disease Control, Lelystad
Drs. C. Pellicaan
Faculty of Veterinary Medicine, University of Utrecht, Utrecht
Dr. W. van Pelt
National Institute for Public Health and the Environment, Bilthoven

The following persons contributed to the writing of MARAN 2004:
Drs. H. van der Zee
Food and Consumer Product Safety Authority, Zutphen
Ing. N Bondt
Agricultural Economics Research Institute, Wageningen:

Members of VANTURES
Ing. N. Bondt
Dr. A. van de Giessen
Dr. B. ter Kuile (secretary)
Dr. D.J. Mevius (chairman)
Drs. C. Pellicaan
Dr. W. van Pelt
Dr. E.E. Stobberingh
Drs. H. van der Zee

People involved in providing data for the surveillance of antimicrobial resistance
Central Institute for Animal Disease Control (CIDC), Lelystad:
Kees Veldman, Marga Japing, Jeannette Wup.

National Institute of Public Health and the Environment (RIVM), Bilthoven:
Wim Wannet, Henny Maas, Wilfrid van Pelt, Arjen van de Giessen, Yvonne van Duynhoven

Faculty of Veterinary Medicine, Pharmacy department, Utrecht:
Chris Pellicaan, Emma de Feijter

Food and Consumer Product Safety Authority (VWA):
Zutphen: Henk van der Zee, C.A.M van Heerwaarden, J.T.M. Zwartkruis
VWA/KvW teamleaders Microbiology: H. van der A, B. Kaandorp, J. Koch, G. van der Vlag, F. van der Zanden
The Hague: Benno ter Kuile

Ministry of Agriculture, Nature and Food Quality:
Max Siemelink

Agricultural Economics Research Institute (LEI), Wageningen:
Nico Bondt, Linda Puister-Jansen

Animal Health Service:
Miriam Koene, Jan Sol

National Inspection Service for Livestock and Meat (RVV):
Ate Jelsma
RVV-Teamleaders: D.A. Edler, N. Zijp, K.J. Pollaris, D.L. Züchner, van Giessen, H.H. van Mierlo, H.J. Schreuder, Mw. A. Nieuwenkamp, P.H.E. Vergunst, T.K. Rasi, J. Veldink, V. Kuske, M.H. Biesheuvel, M.K. Spiegelhof, Mw. Y. Rockland, Mw. H. van bemmel, H. Manni, J. Schiewe, P. Smit.
RVV-Contact persons: W. Koops, H. Hespe, P. van Krieke, H. Derikx, J. Rademakers, Mw. M. Doornbos, Mw. T. Nijbauer, J. de Haan, N. Venselaar, I. Schendel, J. Rademaekers, Mw. I. Hoogendoorn, Mw. C. Tump, T. van der Veer, H. Vrieling, J. Stevens.

Slaughterhouses:
Compaxo Vlees Zevenaar, Dumeco Boxtel, SLH Nijmegen, SLH Eindhoven, Dumeco Helmond, Dumeco Weert, Storteboom Kornhorn, Storteboom Putten, Plukon Wezep, GPS Nunspeet, Pingo Almelo, Goossens Weert, Dumeco Twello

This study was primarily financed by the Ministry of Agriculture, Nature and Food Quality, through project ‘Antimicrobial resistance Research in Animals’, 8021822000, project leader Dr. D.J. Mevius and ‘Monitoring of Antimicrobial Consumption', 30255, project leader Ing. N. Bondt.
The Food and Consumer Product Safety Authority provided additional financing for the work of Drs. H. van der Zee in animal products.

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Summary, Conclusions and Recommendations

Usage of antibiotics
In the last year and in general over the last decade, sales of antibiotics for therapeutic use have increased faster than the number of 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 increase in antibiotic consumption. The data presented here confirm the idea that the quantity and intensity of usage of antibiotics is increasing. 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 shown the highest relative rise in 2004.

An explanation used to justify the growth of the antibiotic sales is the emergence of new infectious diseases in pigs (PIA and Circo-virus). Nevertheless other causes may also be considered. 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. This effect may be reinforced by the economic position of the food animal production sector. Furthermore, over the use of antibiotics no justification has to be made to the authorities and the consumer.

In Dutch broilers quinolones are used frequently, although there is a substantial variation in use between farms. Virtually all flocks are exposed to antibiotics; a substantial part is exposed to quinolones. The quinolone flumequine has the highest contribution. Use of flumequine may also select for resistance to fluoroquinolones (enrofloxacin and difloxacin). Prescribing quinolones by veterinarians is often accompanied by susceptibility testing, however the interpretations of these tests for the antibiotic choice remain unclear. The predominant indications for prescribing quinolones are *E. coli*-infections.

In Dutch pig industry, it is assumed that most antibiotics are used in breeding facilities, whereas the use in fattening facilities was relatively low. As expected most antibiotics in pigs are used as group-medication. Tetracyclines and trimethoprim/sulphonamide combinations are often used in group medication. The breeding facilities use a relatively high amount of broad-spectrum penicillins and trimethoprim/sulphonamides.
In Dutch dairy cattle antibiotics are used less frequently compared to broilers and pigs. Fifty percent of the dosages are administered locally in the udder. Information on usage of antibiotics for therapeutic purposes in veal calves is lacking.

Trends in resistance
In *Salmonella* resistance in the most predominant serovars causing infections in humans (Enteritidis and Typhimurium) remained stable. Resistance levels and multiple resistances were substantially higher in *S.* Typhimurium than in *S.* Enteritidis. High level resistance to fluoroquinolones was observed in a serovar related to travel infections (*S.* Kentucky). The increase in 2003 in nalidixic acid resistant, ciprofloxacin decreased susceptible, *S.* Enteritidis, related to imported contaminated eggs, was not followed by a decrease in 2004.
The prevalence of *S.* Java in broilers, the predominant serovar in these animals, hardly decreased in 2004. The resistance levels in this serovar remained stable.

In *Campylobacter* spp., highest resistance levels were observed in *C. coli* from pigs. Resistance levels to the quinolones were substantially higher in poultry, reflecting the use pattern of this antimicrobial class in these animals. Resistance to erythromycin was only present in *C. coli* and highest in strains from pigs. However, resistance to erythromycin was found in both endemic *C. jejuni* and *C. coli* in

humans and is therefore assumed to be predominantly travel related, related to consumption of contaminated imported products or derived by human therapeutic use of erythromycin.
In *Campylobacter* the prevalence of multiple resistant strains is highest in pigs compared to poultry. A tendency to an increase in resistance can be observed in poultry and for amoxicillin and doxycycline. In pigs for most antibiotics a tendency to increase in resistance is observed. Resistance to nalidixic acid and ciprofloxacin is stable

Data from 2004 in the current report indicate a slow overall increase in resistance in the indicator organisms for the commensal gut flora: *E. coli* and to a lesser extend enterococci. The increase is mainly observed in the older classes of antibiotics (amoxicillin, tetracycline, trimethoprim and sulphonamides). It reflects the tendency of increased usage of antibiotics in food animals since 1998. Resistance to formerly used growth promoters was stable or slowly decreasing (vancomycin). In spite of the increased use of (fluoro)quinolones in 2004, the resistance levels remained stable.
In *E. coli* strains randomly isolated from broiler faeces resistance to cefotaxime increased compared to 2003. This phenomenon is surprising because its occurrence cannot be explained by the use of third-generation cephalosporins in these animals. Other ways of selection must exist.

Comparisons of resistance data from Dutch food-animal sources for both *Salmonella* and *Campylobacter*, indicates than other sources than Dutch food animals contribute to infections with resistant organisms in humans. These sources include travel related infections, but may also include contaminated imported food products. Also antimicrobial therapy of human patients suffering from acute infectious gastro-enteritis cannot be excluded as a contributing factor.
It stresses the necessity for The Netherlands to focus further on imported products in an attempt to quantify the contribution of the imported products to the resistance situation in The Netherlands.

In broilers the resistance levels are higher than in pigs. The first data from veal calf products indicate that in veal calves the level of resistance is similar to broilers. Resistance in food products from sheep, goats, and biologically reared chicken are lowest.

In 2004 human clinical isolates of *Listeria monocytogenes*, a potential zoonotic organism, were included in the surveillance. The vast majority of the strains were susceptible to all antibiotics.

In general *E. coli* strains isolated from milk samples from cows suffering from mastitis were susceptible to the antibiotics included in the panel. The related coliform bacteria (o.a. *Klebsiella, Enterobacter)* showed a high level of resistance to amoxicillin, and to the combination with clavulanic acid. The *S. aureus* isolates tested were susceptible to most antibiotics, except a limited resistance level for penicillin (12.1%). MRSA was not detected in milk. The coagulase negative staphylococci were more resistant than *S. aureus,* 6.1% was *mecA*-positive. In the streptococci only resistance to erythromycin, lincomycin, pirlimycin and tetracycline was observed.

It was surprising that in comparison to previous reports not all *Brachyspira hyodysenteriae* strains were resistant to tylosine (68.8%) in comparison with previous reports. All strains were susceptible to the pleuromutilins.

Mycoplasma synoviae was susceptible to doxycycline and the macrolides, but resistance to enrofloxacin and difloxacin was detected.

Conclusions and recommendations
It can be concluded that therapeutic usage of antibiotics in food animals in The Netherlands steadily increased from 1998 till 2003 followed by a substantial increase in 2004. Determinants for the increase can only be speculated upon, but it seems likely that economic factors are the most important ones. The resistance levels in animal bacteria show a simultaneous tendency to increase. In The Netherlands the Royal Veterinary Association's Antibiotics Policy Working Party published its policy in 1994. Rational and restrictive use of antibiotics was one of the foundations of this policy. Guidelines for therapy (so called formularia) have been developed and their use promoted since the mid nineties of

the last century. Moreover, in the nineties the Ministry of Agriculture published its policy to reduce the amount of veterinary medicinal products use in animals. Data in this report demonstrate that these policies have not totally met their goals. The constant developments in food-animal production warrant an evaluation of the existing policy and its implementation. 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.

Although a direct relation exists between usage of antibiotics in poultry and the occurrence of resistant food borne zoonotic pathogens causing infections in humans (*C. jejuni* resistant to fluoroquinolones), a negative effect on therapy in human patients for this diseases in the Netherlands has not been documented. The first choice drugs for treatment of campylobacteriosis in humans are macrolides, for which in *C. jejuni* from Dutch poultry no resistance was detected but is about 2% in patients with an endemic acquired infection.

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

- Determinants for increased usage of antibiotics in food animals need to be examined
- The validity and the effects of the current antibiotic policy need to be re-evaluated
- Imported food products should be monitored for relevant resistant organisms

Samenvatting, Conclusies en Aanbevelingen

Gebruik van antibiotica
In het afgelopen jaar en zijn algemeenheid in het laatste decennium zijn de hoeveelheden verkochte antibiotica voor therapeutisch gebruik sneller toegenomen dan het aantal landbouwhuisdieren. Dit terwijl de hoeveelheid aan verkochte antimicrobiële groeibevorderaars gestaag afnam. Daar de relatieve bijdrage van iedere therapeutische klasse antibiotica ongeveer gelijk bleef, kunnen verschillen in potentie van gebruikte antibiotica slechts voor een klein deel hebben bijgedragen aan de groei in consumptie van antibiotica. De data die in dit rapport worden gepresenteerd bevestigen dat er een toename bestaat in de hoeveelheid en intensiteit van gebruik van antibiotica. Verkoopscijfers van chinolonen en macroliden (twee antibiotica klassen waarvan het gebruik in dieren ter discussie wordt gesteld in verband met potentiële volksgezondheidsrisico's) gaven de grootste relatieve toename te zien in 2004.

De toename in gebruik kan mogelijk verklaard worden door de toename in infectieziekten bij biggen (PIA en Circo-virussen). Echter ook andere oorzaken kunnen een rol spelen. Vergelijkbaar met andere landen bestaat er in Nederland weinig economische druk ten behoeve van een restrictief antibioticumgebruik. In tegendeel, het antibioticumgebruik geeft economische voordelen voor producenten en dierenartsen en omdat ze relatief goedkoop zijn ook voor de veehouder. Dit in tegenstelling tot duurdere huisvesting en management maatregelen. Dit effect kan worden gestimuleerd door de economische positie van de dierhouderij. Daarnaast speel mogelijk nog een rol het gebruik van antibiotica niet hoeft te worden verantwoordt aan de overheid en de consument.

In Nederlandse vleeskuikens worden chinolonen frequent gebruikt, hoewel het gebruik per veehouder sterk varieert. Bijna alle koppels worden blootgesteld aan antibiotica; een substantieel deel van de koppels wordt blootgesteld aan chinolonen. Het chinolon flumequine wordt het meest frequent gebruikt, dit middel kan ook selecteren voor resistentie tegen de fluorochinolonen (enrofloxacin en difloxacin).

Veterinairen schrijven chinolonen meestal pas voor na een gevoeligheidsbepaling, hoewel niet duidelijk is in hoeverre de interpretatie van deze testen een rol speelde bij de therapiekeuze. De meest voorkomende indicatie voor het toedienen van chinolonen bij pluimvee is colibacillosis.

In de Nederlandse varkenshouderij wordt aangenomen dat het merendeel van het antibioticumgebruik plaatsvindt op vermeerderingsbedrijven, terwijl het gebruik bij mestvarkens relatief gering is. Zoals werd verwacht werd het merendeel van de antibiotica bij varkens als groepsmedicatie toegediend, waarbij tetracyclines en trimethoprim/sulfa combinaties het meest worden gebruikt. Op vermeerderingsbedrijven worden relatief veel breed-spectrum penicillines en trimethoprim/sulfa's gebruikt.
In Nederlands melkvee worden beduidend minder antibiotica gebruikt dan in vleeskuikens en varkens. De helft van alle doseringen worden toegediend in de uier. Informatie over gebruik bij vleeskalveren ontbreekt.

Trends in resistentie
In *Salmonella* bleef het resistentieniveau in de meest voorkomende serovars bij de mens (Enteritidis en Typhimurium) stabiel. In *S.*Typhimurium kwam resistentie en multiresistentie vaker voor dan in *S.* Enteritidis. High level resistentie tegen ciprofloxacin kwam voor in een serovar gerelateerd aan reizen naar Egypte (*S.* Kentucky). De toename in 2003 van nalidixinezuur resistente, ciprofloxacin verminderd gevoelige, *S.* Enteritidis, gerelateerd aan geïmporteerde eieren werd niet gevolgd door een afname in 2004.

Het voorkomen van *S.* Java in vleeskuikens, het predominante serovar in deze dieren, nam nauwelijks af in 2004. De resistentieniveaus bleven gelijk.

In *Campylobacter* spp. werden de hoogste resistentieniveaus bereikt in *C. coli* uit varkens. Resistentie tegen chinolonen kwam meer voor in pluimveestammen, hetgeen een afspiegeling is van het gebruik in die dieren. Resistentie tegen erythromycine kwam alleen voor in *C. coli* en voornamelijk in stammen uit varkens. Echter erythromycine resistentie werd gevonden in endemische *C. jejuni* en *C. coli* stammen uit mensen en is daarom voornamelijk reisgerelateerd, gerelateerd aan gecontamineerde import producten of het gevolg van humane therapie.
In *Campylobacter* kwamen meer multiresistente stammen voor bij varkens dan bij pluimvee. Een toenemende trend in resistentie werd waargenomen voor amoxicilline en doxycycline. In varkens kan voor de meeste antibiotica een toenemende trend in resistentie worden waargenomen. Resistentie tegen de chinolonen is stabiel.

De data in dit rapport uit 2004 laten zien dat er een langzame toename in resistentieniveau bestaat bij de indicator organismen voor de commensale darmflora, *E. coli* en in mindere mate ook voor de enterokokken. Deze trend werd vooral waargenomen voor de oudere klasse antibiotica (amoxicilline, tetracycline, trimethoprim en sulfonamiden). Dit weerspiegelt de toename in gebruik in landbouwhuisdieren sinds 1998. Resistentie tegen de voormalige groeibevorderaars daalde langzaam of is op een stabiel niveau. In tegenstelling tot het toegenomen gebruik van chinolonen in 2004 bleven de resistentieniveaus stabiel.
In *E. coli* stammen op aselecte wijze verzameld uit vleeskuiken feces werd een toename in resistentie gezien tegen cefotaxime in vergelijking met 2003. Dit is een opvallende bevinding omdat in pluimvee geen cefalosporinen worden gebruikt. Dit betekent dat er andere determinanten voor selectie moeten zijn.

Het vergelijken van resistentie data van zowel salmonella's als Campylobacter uit Nederlandse landbouwhuisdieren indiceert dat er andere bronnen bestaan voor infecties met resistente organismen bij mensen. Deze andere bronnen omvatten reisgerelateerde infecties, maar ook besmette geïmporteerde dierlijke producten. Ook therapie van humane gastro-enteritis gevallen kan niet worden uitgesloten als een factor. Het maakt duidelijk dat de noodzaak bestaat om geïmporteerde dierlijke producten in de monitoring te betrekken.

In vleeskuikens komt in de hele lijn meer resistentie voor dan bij varkens. De eerste data van resistentie in vleeskalveren indiceren dat het resistentieniveau overeenkomt met die van de vleeskuikens. Resistentieniveaus in stammen uit kleine herkauwers, en biologische vleeskippen zijn het laagst.

In 2004 zijn klinische isolaten van *Listeria monocytogenes,* een potentieel zoönotisch organisme, onderzocht op voorkomen van resistentie. Bijna alle onderzocht isolaten waren volledig gevoelig voor alle antibiotica.

Voor de mastitisisolaten van melkvee geldt in zijn algemeenheid dat de onderzochte *E. coli* stammen gevoelig waren voor de geteste antibiotica. De verwante coliforme bacteriën (o.a. *Klebsiella, Enterobacter*) waren vaak resistent tegen amoxicilline en de combinatie met clavulaanzuur. De *S. aureus* stammenwaren meestal gevoelig, m.u.v. een beperkt voorkomen van penicilinne resistentie (12.1%). MRSA werd niet in melk aangetoond. De coagulase negatieve stafylokokken waren vaker resistent dan *S. aureus,* 6.1% was *mecA*-positief. In de uierstreptokokkenwerd alleen resistentie tegen erythromycine, lincomycine, pirlimycine en tetracycline gevonden.

Het was opvallend dat de onderzocht *Brachyspira hydoysenteriae* isolaten niet allemaal resistent waren tegen tylosine (68.8%), dit in vergelijking met eerdere publicaties. Alle stammen waren gevoelig voor de plueromutilins.

Mycoplasma synoviae was gevoelig voor doxycycline en macroliden, resistentie tegen enrofloxacin en difloxacin kwam wel voor.

Conclusies en aanbevelingen
Er kan worden geconcludeerd dat het therapeutische gebruik van antibiotica in landbouwhuisdieren in Nederland gestaag toe is genomen sinds 1998 met een piek in toename in 2004. Determinanten voor deze toename zijn niet met zekerheid bekend, maar het lijkt het meest waarschijnlijk dat economische factoren verantwoordelijk zijn. Ook de resistentieniveaus vertonen een toenemende tendens. De Werkgroep Veterinair Antibioticumbeleid van de Koninklijke Maatschappij voor Diergeneeskunde heeft haar beleid in 1994 gepubliceerd. Richtlijnen voor therapiekeuze (formularia) zijn ontwikkeld en hun gebruik gestimuleerd sinds het midden van de negentiger jaren van de vorige eeuw. Daarnaast voerde LNV in de negentiger jaren een beleid gericht op een algemene reductie van diergeneesmiddelengebruik. De data in dit rapport maken duidelijk dat de doelen van het voormalige beleid niet zijn bereikt. De constante ontwikkelingen in de veehouderij maken het noodzakelijk dat het bestaande beleid en de implementatie dient te worden geëvolueerd.
Momenteel is men bezig met het implementeren van de Directive 2004/28/EU aangaande de 'community code' voor diergeneesmiddelen in de Nederlandse wetgeving. Dit zou ook kunnen worden gebruikt voor het stimuleren van meer selectief en restrictief antibioticumgebruik.

Hoewel een directe relatie bestaat tussen het gebruik van antibiotica in pluimvee en het voorkomen van resistente voedselpathogenen bij humane infecties (*C. jejuni* resistent tegen fluorochinolonen) is een negatief effect op de behandeling van deze infecties bij de mens in Nederland niet gedocumenteerd. De eerste middelen voor behandeling van campylobacteriosis bij de mens zijn macroliden, waartegen in *C. jejuni* uit Nederlands pluimvee geen resistentie is waargenomen.

Gebaseerd op de data in dit rapport kan het volgende worden aanbevolen:
- Determinanten voor de toename in het gebruik dienen te worden onderzocht
- De validiteit en de effecten van het huidige antibioticumbeleid dienen te worden geëvalueerd
- Geïmporteerde dierlijke producten dienen te worden onderzocht op het voorkomen van relevante resistente organismen.

I Usage of antibiotics in animal husbandry in the Netherlands

Highlights
In 2004 the total sales of antibiotics for therapeutic purposes in the Netherlands increased by 59.000 kg (+15%) to 453.000 kg. Total live weight production of the most important users (pigs, broilers and veal calves) increased in this period by 6%. As from 1998 till 2004 the total sales of antibiotics for therapeutic use has increased with 127.000 kg, every year sales have grown faster than the production of animals. In this period the sales of antimicrobial growth promoters have declined by 175.000 kg.

In Dutch poultry antibiotics for therapeutic purposes are mainly used in turkeys and broilers. The usage in laying hens is relatively low. In broilers quinolones are used frequently, although there is a substantial variation between farms. Virtually all flocks have been exposed to antibiotics; a substantial part has been exposed to quinolones or fluoroquinolones. Prescribing quinolones or flouroquinolones by veterinarians is often accompanied by susceptibility testing, however the implications of these tests for the antibiotic choice remain unclear. The predominant indications for prescribing quinolones and fluoroquinolones are *E. coli*-infections.

Most antibiotics for therapeutic purposes in pigs are used as group-medication. Tetracyclines and trimethoprim/sulphonamide combinations are most often used. Most antibiotics are used in breeding facilities (piglets and sows). In dairy cattle antibiotics are used less frequent compared to broilers, turkeys and pigs.
Information on usage of antibiotics for therapeutic purposes in veal calves is lacking.

Usage of antimicrobial growth promoters (AGPs) and coccidiostats

In the Netherlands, manufacturing, distributing and selling of animal feed containing (AGPs) and coccidiostats is in the hands of the feed industry and is not controlled by veterinarians. In 1998 250.000 kg of antibiotics were used as AGPs in the Netherlands. Since cross resistance occurs between antibiotics formerly used as AGP and antibiotics used therapeutically for animals and humans, the use of antibiotics as AGP is put under pressure. Since 1999 only few antibiotics are still allowed and used as AGP. These are flavophospholipol (a glycolipid), avilamycin (an orthosomycin), salinomycin and monensin (ionophores). The latter are used both as AGP and as coccidiostat. The prohibition of the remaining antibiotics as from January 2006, completes the EU drive to phase out all AGPs from livestock production. Based on data provided by feed additive manufacturers it is calculated that the use of AGPs in 2004 was 75.000 kg and remained unchanged compared to 2003. This is a reduction of 70% compared to 1998.

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 generously provided by the FIDIN (manufacturers and importers of veterinary medicines in the Netherlands). In table 1 most recent data (2004) are shown. Sales from 1997 to 2004, 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 (2004) from the agricultural census are shown. In table *3* live weight production[1] (2004) is reported. Livestock statistics over a longer period (from 1997 to 2004) are summarized in figure 2 and figure 3.

[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%.

After a year of stable antibiotic sales in 2003, the total sales of antibiotics increased in 2004 by 59.000 kg (+15%) to 453.000 kg (table 1). This was mainly attributed to an increase in tetracycline sales by 42.000 kg (+19%). Expressed in percentages, the sales of quinolones and fluoroquinolones (+40%, 2.000 kg) and macrolides (+33%, 6.000 kg) increased most rapid.

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 2004 (Statistics Netherlands, CBS) the number of pigs remained more or less unchanged *(*table 2*)*. The Product Boards for Livestock, Meat and Eggs (PVE) however concludes, based on sampled data, that the pig population increased in the second half of 2004 by 3,5 %. Pig live weight production (based on the number of pigs) increased by 0,5 % in 2004 compared to 2003 according to PVE. The broiler population recovered slightly from the avian influenza outbreak in the Netherlands in 2003, the number of broilers increased by 4,7 % (table 2), the live weight production increased by 15%, this is still 12% less than the live weight production in 2002. The veal calf population increased by 4,5% (table 2).

As from 1997, total sales of antibiotics for therapeutic use have increased from 332.000 kg to 453.000 kg in 2004 (+36 %) (figure 1). The veal calf population over this period increased by 8,6 %. The broiler population slightly decreased by 1,7 %. The pig population decreased over this period by 21% (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. From 1998 the total live weight production of pigs, veal calves and broilers decreased by 11,2% (figure 3). It can be calculated that therapeutic antibiotic usage per kg live weight production in 1998 was 0,094 mg, this figure gradually increased to 0,147 mg in 2004 (figure 4). The total Dutch usage per kg live weight production thus calculated, cannot be related to the individual (pig, veal calf or poultry) industries. Apart from an intrinsic higher usage of antimicrobials other variables can also influence this figure. The number of piglets exported has increased in time, piglets are considered to be intensive users of antimicrobials compared to older animals. Another factor influencing this figure is that the relative contribution of veal calves has increased.

In general the relative contribution of different therapeutic groups of antibiotics to total sales has remained stable over the years. In 2004 tetracyclines and trimethoprim/sulphonamide combinations represented 80% of the weight of total sales in antibiotics; in 1997 both classes represented 75%.

Table 1. Total sales of antimicrobials in 2004 in the Netherlands.

Therapeutic group	kg of active substance in 2004 (x1000)	Difference with 2003
Penicillins/cephalosporins	45	18 %
Tetracyclines	269	19 %
Macrolides	24	33 %
Aminoglycosides	9	0 %
Quinolones and fluoroquinolones	7	40 %
Trimethoprim/sulphonamides	93	3 %
Other	6	- 14 %
Total	**453**	**15 %**

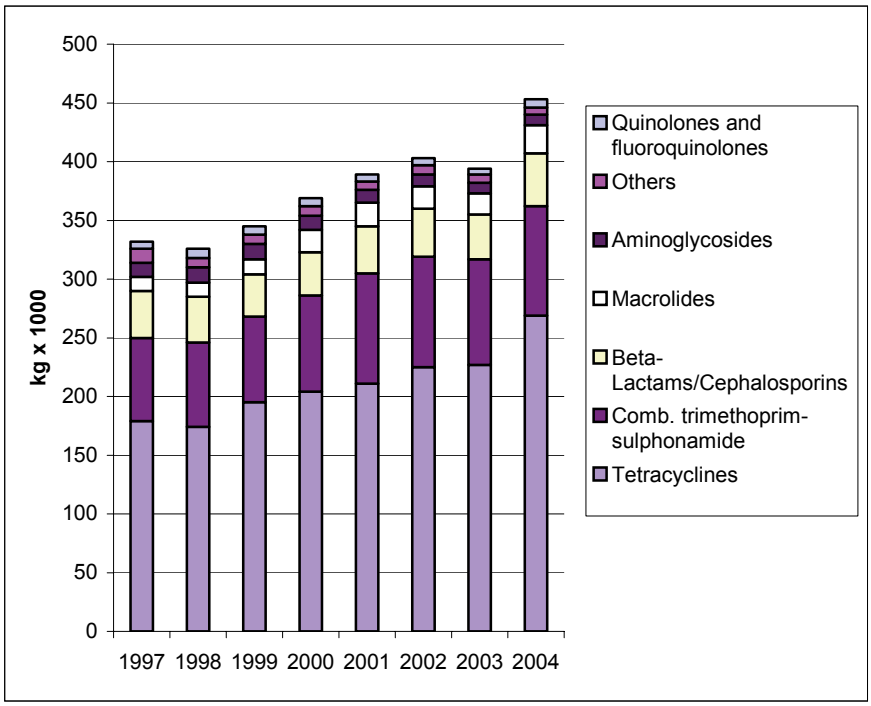
Source: FIDIN.

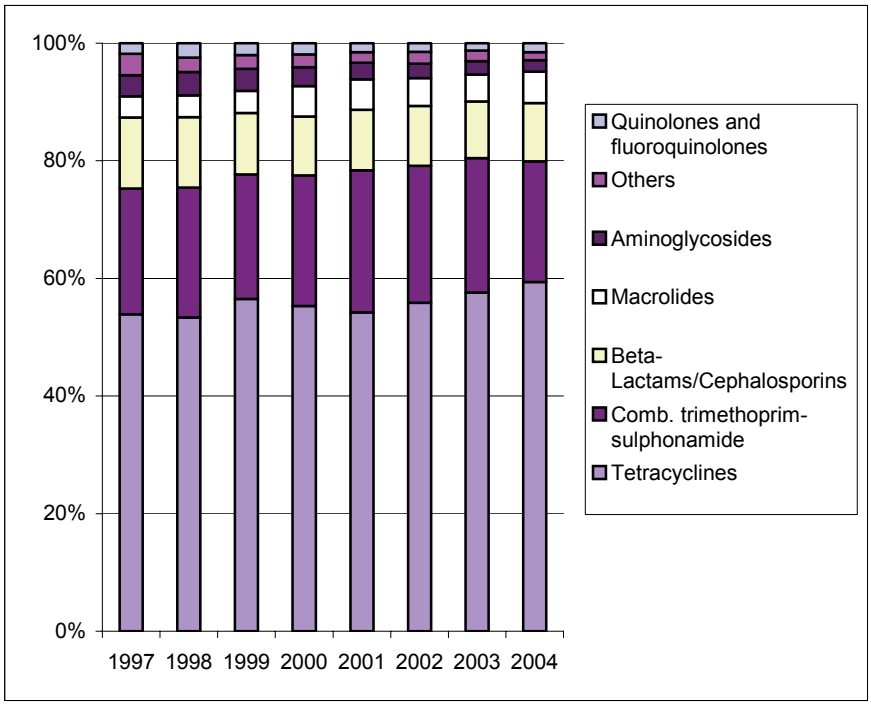
Table 2. Agricultural census in the Netherlands (2004), numbers x 1000

Animal species	N x 1000 in 2004	Difference with 2003 (%)
Dairy cattle	2.636	-0,9
Veal calves	765	4,5
Cows for fattening and grazing	366	0,0
Cattle total	**3.767**	**0,2**
Pigs for fattening (>20kg)	5.382	0,3
Piglets	4.524	-0,4
Pigs other	1.246	-1,1
Pigs total	**11.152**	**-0,2***
Broilers	44.262	4,7
Laying hens	35.668	17,0
Broilers, breeding	5.886	-8,7
Ducks and Turkeys	2.199	10,1
Poultry total	**88.015**	**8,4**
Sheep	1.236	4,3
Rabbits	348	7,1
Goats	282	2,9
Horses and Ponies	129	2,4

Source: Agricultural census, Statistics Netherlands (CBS).
*** Based on sampled data over 2004 PVE concludes that the pig population increased in the second half of 2004 by 3,5 %.**

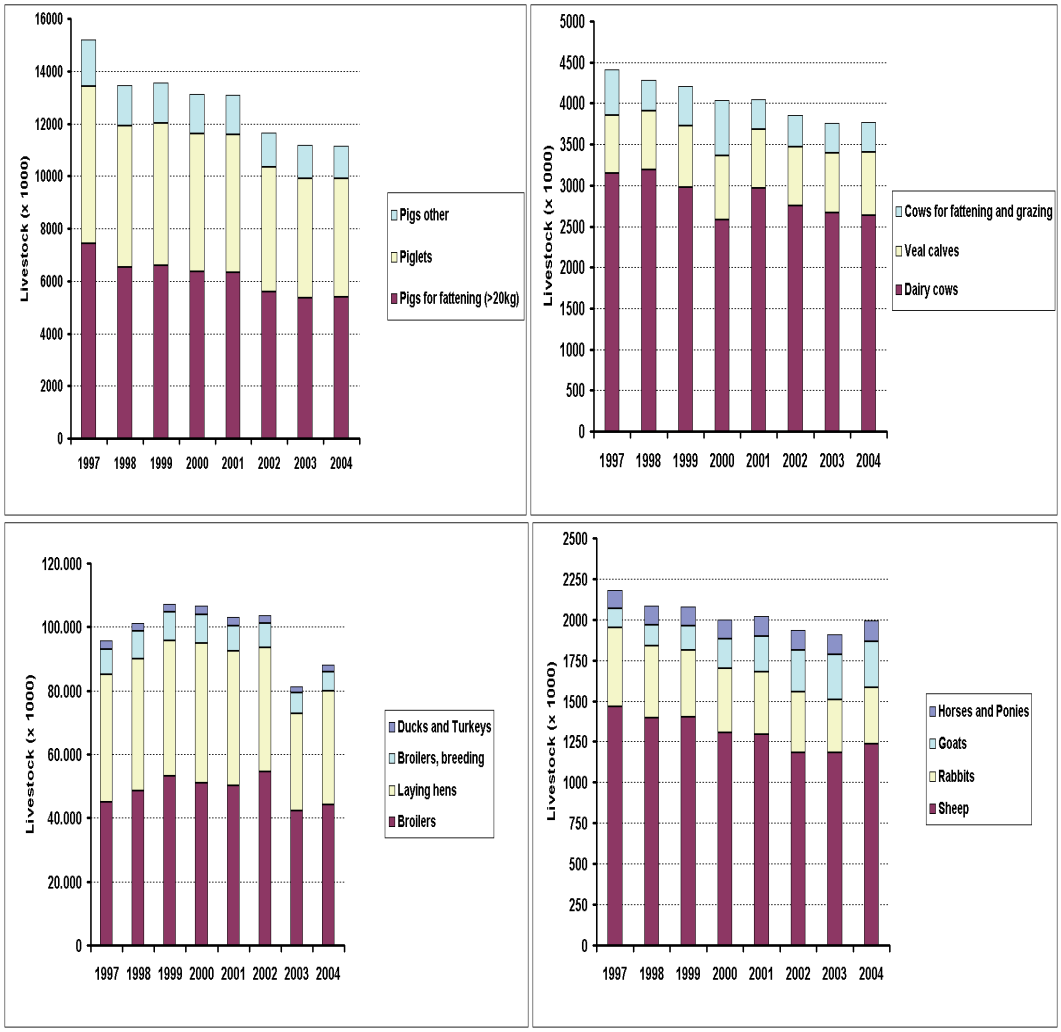
Figure 1. Usage of antibiotics for therapeutic use (active ingredient x 1000 kg) in the Netherlands and the usage expressed as percentages of the total use (relative use) from 1997-2004.





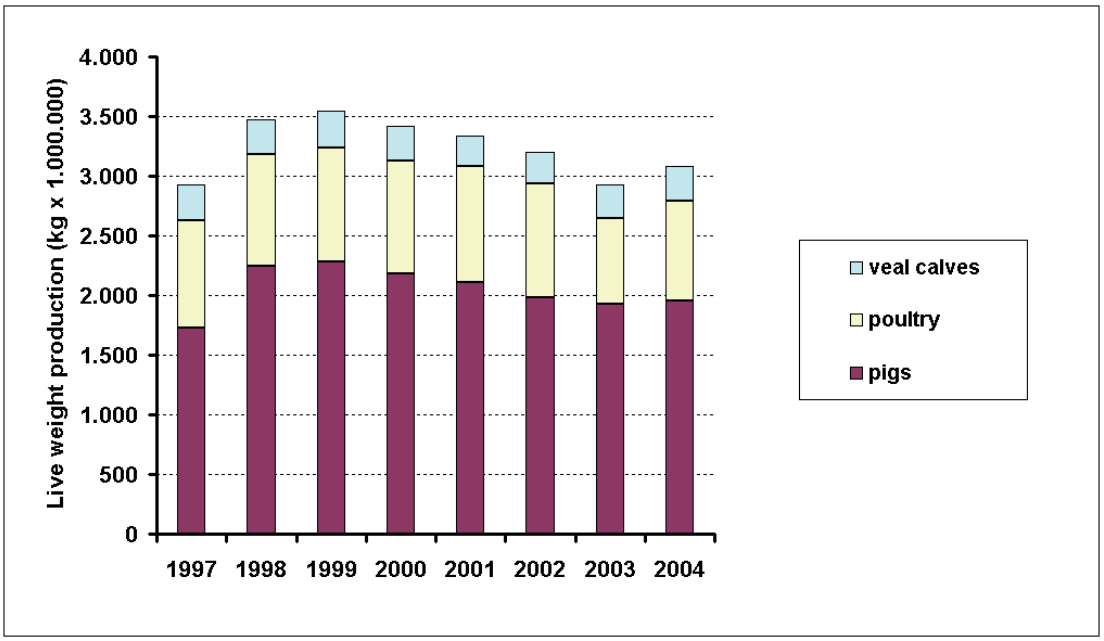
Source: FIDIN

Figure 2. Developments in livestock (x 1000) in the Netherlands 1997 - 2004.



Source: Agricultural census, Statistics Netherlands (CBS).

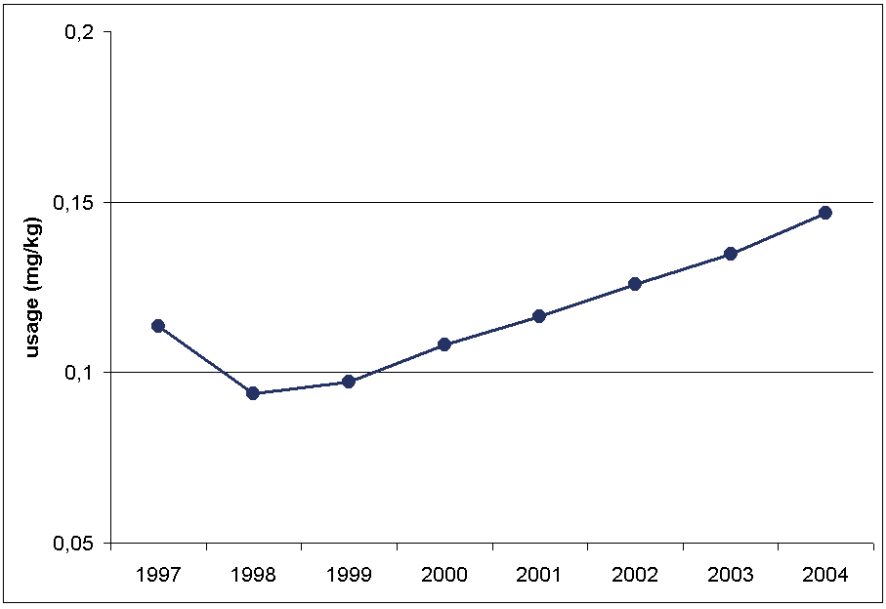
Figure 3. Live weight production in the Netherlands 1997 - 2004.



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 therapeutic usage of antibiotic (mg) per kg live weight production (pigs, poultry and veal calves) 1997-2004



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

The above-mentioned sales data from the pharmaceutical industry offer a general overview on antibiotic usage in the Netherlands. Nevertheless, to obtain more detailed information, a continuous programme 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. It is difficult to obtain information from farm accountancies concerning medicines processed into animal feed by feed mills, as the medicine invoice may originate from the feed mill. Thus it is possible that a part of the in-feed medication is hiding from the observations by LEI and the data in group medication are underestimated.

Table 3. Characteristics of farms and animals included

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		46		Milking cows	3.864	0,3 % (1.471.000)
Pigs		68		Sows	17.875	1,9 % (954.000)
				Fattening pigs (> 20 kg)	73.016	1,4 % (5.383.000)
	Breeding		28			
	Fattening		18			
	Combined		22			
Broiler		15		Broilers	838.000	1,9% (44.262.000)

The data of the first year (2004) are based on 129 farms (table 3): 46 dairy farms, 68 pig farms and 15 broiler farms. The pig farms are divided in breeding (sows and piglets), fattening and closed facilities (breeding and fattening).

In table 4 the number of doses per animal year is presented for non-systematic treatment of dairy cattle (for an explanation of the unit of measurement; see box 1). 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. Per average milking cow 1,70 times a year an antibiotic is administered intramammary during lactation. The combination of amoxicillin with clavulanic acid is used most frequent. Antibiotics to prevent infections during the dry period are administered 1,98 times a year. Considering a preventive treatment in all quarters, a calving interval of 420 days and a 25% culling rate, it can be calculated that 77% of all dry cows is treated with intramammaries. The most frequently used antibiotic is cloxacilline. The antibiotics used in intra-uterine therapy are tetracyclines and cefapirine.

The quantity of antibiotics used for systemic treatment in dairy cattle and their calves is presented in table 5. By parenteral and oral administration 2,40 dd/ay are administered. The use of fluoroquinolones and macrolides is limited. The relative frequent use of the third generation cephalosprins is related to the zero withdrawal time for milk of ceftiofur.

Box 1. 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, the antibiotic usage in pig farm is substantially higher compared to dairy cattle (table 4). Exposure to antibiotics is concentrated in breeding facilities rather than in fattening facilities. Furthermore, in the breeding facilities the number of daily dosages is calculated over the total average weight of sows and piglets (and other pigs) present at a farm. We suppose however that to piglets antibiotics are administered more intensively 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 be higher as calculated here. In particular trimethoprim/sulphonamide combinations and penicillins are used more intensively in breeding facilities (figure 5). This may be related to usage in weaning piglets. Overall, tetracyclines are used often whereas the use of quinolones or fluoroquinolones is limited.

Antibiotic usage in broiler farms is presented in table 7. Broilers in this sample used 16, 3 daily dosages per animal year. This equals to 0,04 dosages per day. During their approximately 40-day live the average broiler in this sample was medicated with antibiotics for therapeutic purposes during 2 days. Tetracyclines (33%) as well as quinolones and fluorquinolones (22%) and trimethoprim/sulphonamide combinations (20%) are used relatively frequent. Fluoroquinolones (enrofloxacin and difloxacin) are used in 1,5 % of all daily dosages. Antibiotic usage (figure 6) and quinolone usage (figure 7) was also measured at flock level. Virtually all flocks (48 out of 51) investigated (94 %) were exposed to antibiotics. In the sample 12 flocks out of 51 flocks (24%) were exposed to quinolones or fluoroquinolones.

Table 4. Number of daily dosages per animal year (dd/ay) administered in dairy cattle (non-systemic use), continuous monitoring programme

Therapeutic Group	Intramammary use in lactating cows	dd/ay
Cephalosporins	Cefoperazone	0,16
	Cefquinome	0,27
Lincosamides	Pirlimycine	0,02
Combinations	Dihydrostreptomycin-benzylpenicillin-nafcillin	0,17
	Neomycin-benzylpenicillin	0,02
	Amoxicillin-clavulanic acid	0,71
	Ampicillin-cloxacillin	0,09
	Lincomycin-neomycin	0,25
	Total milking cows	**1,70**

Therapeutic Group	Intramammary use in dry cows	dd/ay
Penicillines	Cloxacillin	0,80
Combinations	Dihydrostreptomycin-benzylpenicillin-nafcillin	0,31
	Neomycin-benzylpenicillin	0,38
	Ampicillin-cloxacillin	0,49
	Total dry cows	**1,98**

Therapeutic Group	Intra-uterine use in cows	dd/ay
Cephalosporins	Cefapirin	0,06
Tetracyclines	Oxytetracycline	0,13
	Tetracycline	0,01
	Total intra-uterine	**0,20**

Table 5. Number of daily dosages per animal year (dd/ay) administered in dairy cattle and their calves (oral and parenteral administration), continuous monitoring programme

Therapeutic group	Active substance (administration)	dd/ay
Cephalosporins	Cefquinome	0,04
	Ceftiofur	0,27
		0,31
Penicillines	Benzylpenicillin	0,33
	Ampicillin	0,09
		0,42
Macrolides	Erythromycin	0,03
	Tylosin	0,01
		0,04
Fluoroquinolones	Danofloxacin	0,01
	Enrofloxacin	0,04
		0,05
Sulphonamides and trimethoprim	Trimethoprim-sulfadiazine	0,11
	Trimethoprim-sulfadoxine	0,12
	Trimethoprim-sulfamethoxazole	0,01
	Sulfadimidine	0,01
		0,25
Tetracyclines	Doxycycline	0,04
	Oxytetracycline	0,53
		0,57
Others	Florfenicol	0,01
	Lincomycin	0,03
	Colistin	0,28
		0,32
Combinations	Ampicillin-colistin	0,01
	Amoxicillin-colistin	0,01
	Dihydrostreptomycin-benzylpenicillin	0,07
	Lincomycin-spectinomycin	0,01
	Neomycin-benzylpenicillin	0,32
		0,42
	Total	**2,38**

Table 6. Average number of daily dosages per animal year (dd/ay) administered as group medication or individual (ind.) medication in three types of pig farms, continuous monitoring programme

Therapeutic group	Active substance	Fattening facilities		Breeding facilities (sows and piglets)		Combined facilities (breeding and fattening)	
		Group*	Ind.	Group*	Ind.	Group*	Ind.
Penicillines	Benzylpenicillin	-	0,24	-	0,61	-	0,53
	Ampicillin	-	0,18	0,27	0,54	0,89	0,26
	Amoxicillin	0,01	0,01	1,72	0,12	1,16	0,06
	Total	**0,01**	**0,43**	**1,99**	**1,27**	**2,05**	**0,85**
Cephalosporines	Cefquinome	-	-	-	0,01	-	0,01
	Ceftiofur	-	-	-	0,01	-	0,03
	Total	**-**	**-**	**-**	**0,02**	**-**	**0,04**
Macrolides and lincosamides	Tilmicosin	0,02	-	0,23	-	0,22	-
	Tylosin	0,55	0,04	0,06	0,01	0,45	0,01
	Lincomycin	0,01	-	-	-	-	-
	Total	**0,58**	**0,04**	**0,29**	**0,01**	**0,67**	**0,01**
Quinolones	Flumequine	-	-	0,22	-	-	-
	Enrofloxacin	-	0,01	-	0,02	-	-
	Total	**-**	**0,01**	**0,22**	**0,02**	**-**	**-**
Sulphonamides and trimethoprim	Tmp-sulfadiazine	0,64	-	4,46	0,12	1,20	0,01
	Tmp-sulfadoxine	-	-	-	0,18	-	0,03
	Tmp-sulfamethoxazole	0,61	-	2,61	0,01	2,95	-
	Total	**1,25**	**-**	**7,07**	**0,31**	**4,15**	**0,04**
Tetracyclines	Doxycycline	3,46	-	4,57	-	7,39	-
	Oxytetracycline	6,73	0,47	4,40	0,26	4,55	0,29
	Total	**10,19**	**0,47**	**8,97**	**0,26**	**11,94**	**0,29**
Aminoglycosides	Gentamicin	-	-	0,03	0,01	0,05	-
	Total	**-**	**-**	**0,03**	**0,01**	**0,05**	**-**
Combinations	Lincomycin-spectinomycin	0,05	-	0,54	0,02	0,01	-
	Amoxicillin-colistin	0,07	0,02	0,04	0,09	-	0,07
	Neomycin-benzylpenicillin	-	-	-	0,02	-	0,10
	Dihydrostreptomycin-benzylpenicillin	-	0,21	-	0,74	-	0,70
	Total	**0,12**	**0,23**	**0,58**	**0,87**	**0,01**	**0,87**
Others	Tiamulin	-	-	0,02	-	-	-
	Colistin	0,13	-	1,57	-	0,36	-
	Florfenicol	-	0,01	-	0,03	-	0,03
	Total	**0,13**	**0,01**	**1,59**	**0,03**	**0,36**	**0,03**
Total		**12,28**	**1,19**	**20,74**	**2,79**	**19,23**	**2,13**

* It is possible that a part of the in-feed medication is hiding from the observations by LEI and therefore the data on group medication in pigs may be underestimated.

Table 7. Average number of daily dosages per animal year (dd/ay) administered in broiler farms, continuous monitoring programme

Therapeutic group	Active substance	dd/ay
Penicillines	Ampicillin	0,41
	Amoxicillin	2,60
	Total	**3,01**
Macrolides and lincosamides	Tylosin	0,73
Quinolones	Enrofloxacin	0,25
	Flumequine	3,40
	Total	**3,65**
Sulphonamides and trimethoprim	Trimethoprim-sulfachloorpyridazine	1,57
	Trimethoprim-sulfamethoxazole	1,69
	Sulfadimidine	0,07
	Total	**3,33**
Tetracyclines	Doxycycline	4,76
	Oxytetracycline	0,84
	Total	**5,60**
Aminoglycosides	Neomycin	0,45
Combinations	Lincomycin-spectinomycin	0,04
Total		**16,81**

Figure 5. Use of antibiotics for therapeutic use in different types of pig farms

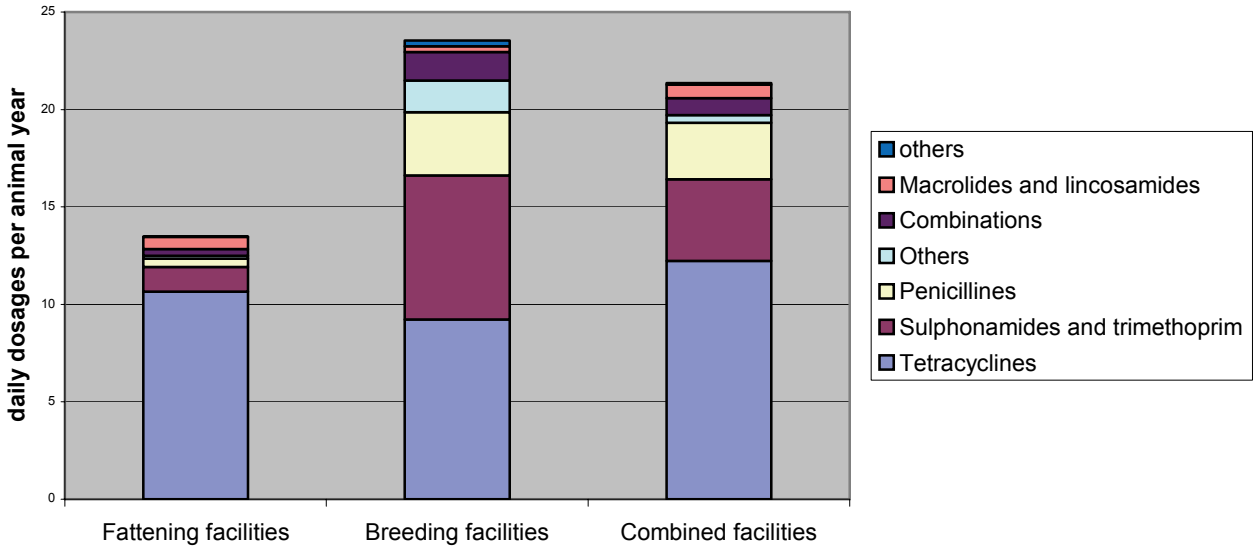


Figure 6. Antibiotic usage in broilers (number of daily dosages per animal year (dd/ay)) per flock

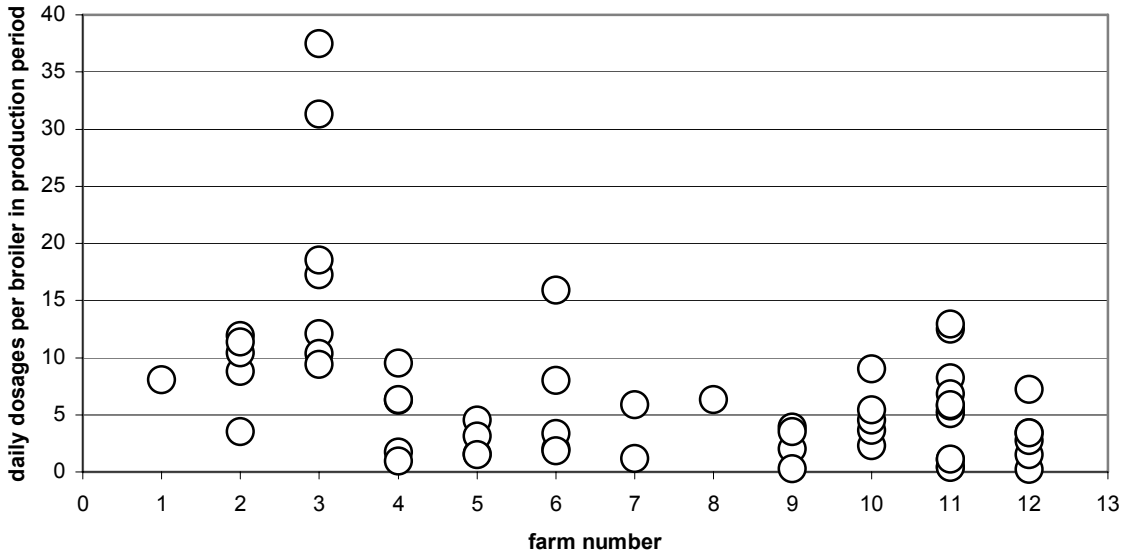
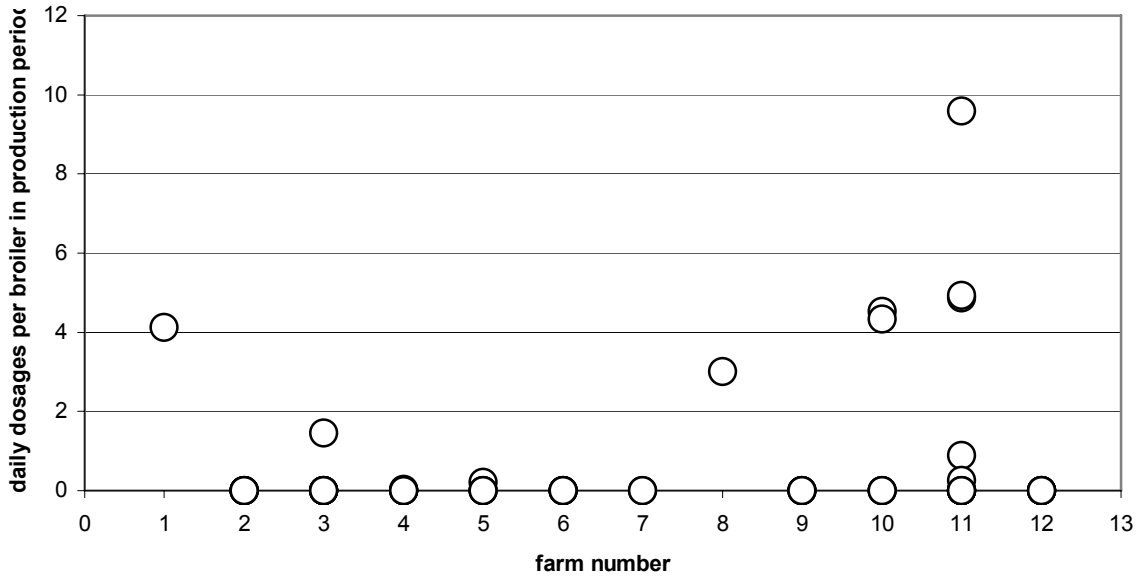


Figure 7. Quinolone usage in broilers (number of daily dosages per animal year (dd/ay)) per flock



Usage of antibiotics, specifically quinolones and fluoroquinolones, in poultry

Although the use of quinolones and fluoroquinolones in poultry is under debate, little is known about the quantity and the way antibiotics are used in these animals. Results from a study on antibiotic usage usage in poultry in 2004, are presented here. Antibiotic prescription data from 7 volunteering veterinary practices, specialized in poultry were sampled and analyzed. Livestock from these practices amounted to 13,3 million animals in 345 farms (15% of the total Dutch poultry population). The period examined was January 2004 till October 2004. In this period antibiotics were prescribed 1525 times by the participating practitioners.

In table 8 the number of doses per animal year is presented (for an explanation of the unit of measurement: see box 1). As expected, antibiotics were prescribed most frequently to broilers and turkeys. The intensity of antibiotic usage in turkeys and broilers is comparable. Turkeys however live longer and consequently individual turkeys are exposed more frequently to antibiotics.

Broilers were prescribed 21,6 daily dosages per animal year (dd/ay). This equals to 0,06 daily dosages per animal day (dd/ad). During their approximately 40-day live the average broiler in the sample was medicated with antibiotics for therapeutic purposes during 2 - 3 days. In this sample the most commonly used antibiotics in broilers were tetracyclines (35 % of the dosages) and the first generation quinolone flumequine (34 %). In broilers the fluoroquinolones (enrofloxacin and difloxacin) were used in 2 % of the dosages. A notable variation in antibiotic usage was found between farms. Six broiler farms did not use any antibiotics, whereas the highest user was 74 dd/ay. No relation was found between the number animal in broiler farms and the amount of antibiotics prescribed (figure 8). Assuming that quinolones are used during three consecutive days and that, if broilers are treated with quinolones, they are treated only once in a life-time, it can be calculated that out of 100 flocks slaughtered, to 27 of them quinolones or fluoroquinolones were administered.

In turkeys, tetracyclines are commonly used (73 % of the dosages). As flumequine is not approved for use in turkeys, its use is limited (0,3% of the total number of dosages). Due to the high price-level the use of fluoroquinolones in turkeys is also low (0,7%). The amount of antibiotics prescribed to laying hens is limited. In turkeys and broilers the withdrawal time only has to be considered before slaughter. In laying hens every antibiotic treatment has economic consequences, since the eggs cannot be used in human consumption during the withdrawal time. The way laying hens are housed (battery cages, free-range system, aviary housing system and organic poultry farming) did not have a significant influence on antibiotic usage.

The indications for prescribing quinolones and fluoroquinolones in poultry are reflected in figure 9. Most frequent reasons for prescribing are *E. coli*-infections (i.e. yolk sac infections, peritonitis, synositis, airway infections, infections of the locomotion system). When prescribing quinolones or fluoroquinolones in poultry, in 71% of the cases a susceptibility test was performed. The quality and judgment of the test, as well as the follow up actions are not evaluated.

Table 8. Average number of daily dosages per animal year (range),

	Turkeys	Laying hens (consumption eggs)	Laying hens (hatching eggs)	Breeding animals	broilers
Penicillines (broad spectrum)	2,4 (0 – 9,6)	0,1 (0 - 3,4)	0,4 (0 – 27,7)	1,3 (0 – 10,6)	2,6 (0 – 12,1)
Macrolides	2,2 (0 – 7,7)	0 (0 – 2,0)	0 (0 – 0,1)	0,0 (0 – 1,1)	1,2 (0 – 9,3)
Third generation quinolones (enrofloxacin and difloxacin)	0,7 (0 – 2,0)	0 (0 - 0,1)	0,1 (0 – 3,6)	0,1 (0 – 2,6)	0,5 (0 – 13,0)
First generation quinolones (flumequine)	0,3 (0 – 2,9)	0,2 (0 – 6,8)	-	0,2 (0 – 4,6)	7,3 (0 –51,1)
Sulfonamides	0,7 (0 – 4,48)	0 (0 - 2,0)	-	-	0,1 (0 – 4,6)
Sulphonamides and trimethoprim	0,0 (0 – 0,15)	0 (0 –0,5)	-	0,6 (0 – 9,3)	1,8 (0 – 24,6)
Tetracyclines	18,2 (0 – 42,3)	0,7 (0 – 4,6)	1,3 (0 – 20,7)	0,8 (0 – 12,6)	7,5 (0 – 12,1)
Aminoglycosides	0,5 (0 – 4,3)	0,4 (0 – 27,7)	1,0 (0 – 14,6)	0,1 (0 – 3,5)	0,4 (0 – 9,8)
Polymyxines	-	-	-	-	0,0 (0 – 2,0)
Spectinomycin in combination with clindamycin and lincomycin	-	0 (0 – 0,1)	-	-	0,1 (0 – 5,4)
Total	**25,0**	**1,4**	**2,8**	**3,1**	**21,6**

Figure 8 Relation between farm-size and antibiotic usage

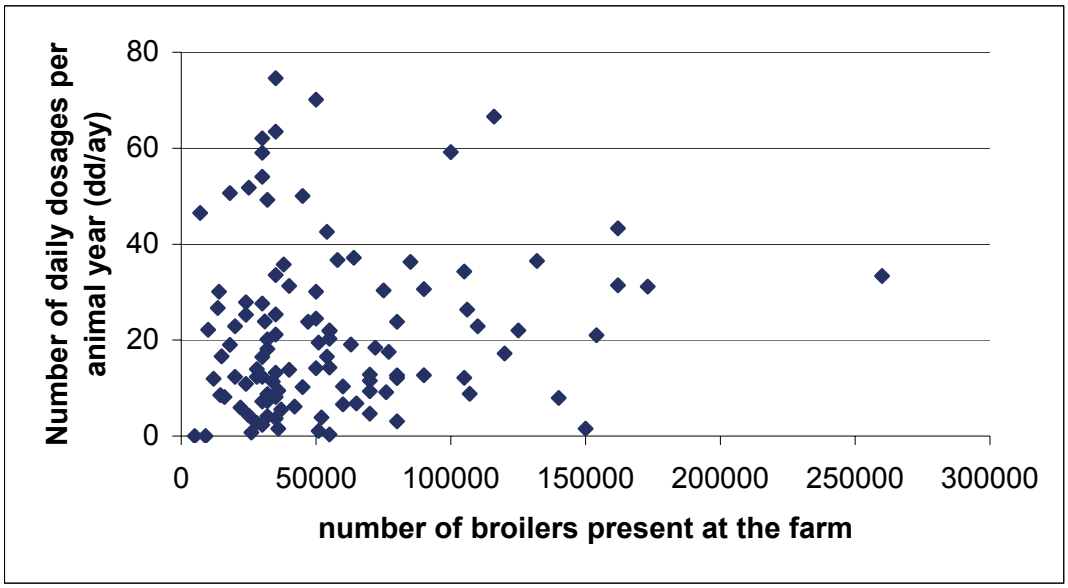
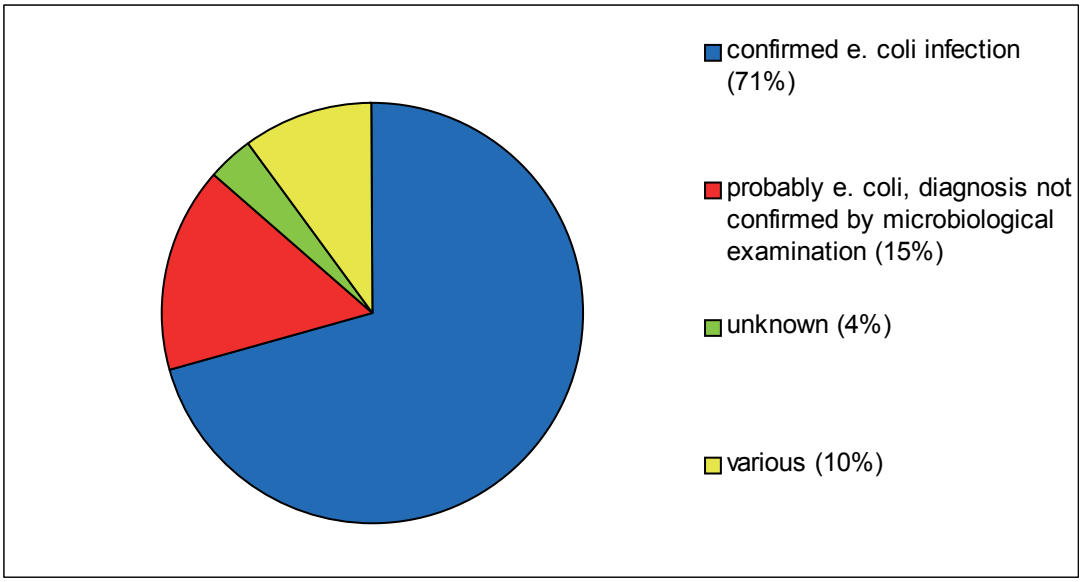


Figure 9. Indications for quinolone prescriptions in poultry



II Resistance data

In this chapter susceptibility test results are presented as determined in 2004 for the food-borne pathogens *Salmonella, Campylobacter* and *Escherichia coli* O157, the food-borne commensal organisms *E. coli*, *Enterococcus faecium* and *E. faecalis*, *Listeria monocytogenes* the bovine mastitis pathogens *Staphylococcus aureus, Streptococcus uberis, S. dysgalactiae, E. coli* and coliform bacteria, poultry pathogens *Mycoplasma synoviae* and pig pathogens *Brachyspira hyodysenteriae.*
MIC-data on the bovine respiratory disease pathogens *Pasteurella multocida* and *Mannheimia haemolytica* will not be included in this report because the number of strains isolated in 2004 was too small.

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 2004 *S.* Enteritidis was still the most prevalent serovar in humans, but the incidence decreased compared to 2003. *S.* Typhimurium was the second most prevalent serovar in humans. Pigs and cattle were the most important animal sources of *S.* Typhimurium, and layers (eggs) of *S.* Enteritidis. In broilers *S.* Java was isolated most frequently. In these animals *S.* Enteritidis and *S.* Typhimurium constitute only a small fraction of all salmonella's.
Resistance levels and multiple resistances were substantially higher in *S.* Typhimurium than in *S.* Enteritidis. Resistance to ciprofloxacin was incidentally detected in *S.* Kentucky strains isolated from human patients (also detected in 2002 and 2003). These strains were related to travel to Egypt and not to Dutch food-animals. Resistance to nalidixic acid was more commonly present in *S.* Enteritidis and *S.* Typhimurium isolated from humans than from animals. It was predominantly present in *S.* Enteritidis (Pts1, 6a, and 8), *S.* Hadar, *S.* Virchow and *S.* Java and only rarely in *S.* Typhimurium. It can be concluded that nalidixic acid resistant strains of *S.* Enteritidis and *S.* Typhimurium isolated from humans either originate from imported animal products or from travel related infections. Therapy of humans may have contributed as well.
The prevalence of *S.* Java in broilers slightly decreased in 2004. However, at retail the proportions of poultry meat products contaminated with *S.* Java remained at the same high level. The resistance levels in this serovar remained stable.

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 and food-animals and animal feeds (table 9). In 2004, like in former years, *S.* Typhimurium and *S.* Enteritidis were by far the most frequently isolated serovars of *Salmonella* in humans in The Netherlands. In pigs *S.* Typhimurium and in cattle *S.* Typhimurium and *S.* Dublin were the most prevalent serovars. In poultry a difference existed in prevalence of *Salmonella* spp. between broilers and layers. In broilers *S.* Paratyphi B var. Java (*S.* Java) and *S.* Infantis, and in layers *S.* Enteritidis and *S.* Senftenberg were the predominantly isolated serovars.
Travel contributed from 0% to 50% of the cases of human salmonellosis depending on the sero/phagetype. Among the most frequently isolated human serovars travel contributed substantially more to the incidence of *S.* Enteritidis than for *S.* Typhimurium.

In 2004 the incidence of *S.* Enteritidis slightly decreased again after the sudden increase in 2003 related to the increase of imported eggs. The occurrence of *S.* Java in broilers decreased. The increase in prevalence of *S.* Infantis and *S.* Senftenberg in layers in 2003 is followed by a decrease for *S.* Infantis in 2004 and a substantial further increase in prevalence for *S.* Senftenberg. However, this is

not relevant for public health because Dutch layers are not the source for infections with *S.* Senftenberg.

Table 9. Most prevalent *Salmonella* sero-, and phagetypes isolated in 2004 (2003 between brackets) from humans, pigs, poultry, broilers and layers[2] and the % travel related infections in 2003 – 2004.

		Humans	Pigs	Cattle	Poultry	Broilers	Layers
Total number sent to RIVM		**1626**	**388**	**187**	**568**	**355**	**141**
Sero/phage type	**% Travel**	**% of the total sent to RIVM in 2004 (2003 data between brackets)**					
Typhimurium	2%	28.5	47.9	15.0	3.5	3.9	2.8
DT104	2%	6,5(7,4)	11,8(17,3)	4,8(8,4)	0.9	1.1	0.7
ft 507	1%	6.4	12,4(9)	3.2	0.2	0.3	--
ft 508	2%	1,7(1,1)	2,3(1,1)	--	0.5	0.8	--
ft 510	7%	1.4	1.5	0.5	--	--	--
ft 90	0%	0.1	4,1(0)	1.1	--	--	--
ft 655	3%	1.1	--(2,9)	0.5	--	--	--
ft 295	0%	0.2	--(0,7)	--(1,7)	--	--	--
ft 350	0%	0.1	1,5(0)	--	--	--	--
Enteritidis	9%	47,2(55,2)	--	--	9.5	5.9	22.0
Pt 4	7%	13,8(19)	--	--	3.9	2.3	8,5(12,6)
Pt 1	17%	5,2(7,9)	--	--	0.2	0,3(1)	--(0,8)
Pt 21	7%	8,7(12,2)	--	--	1,6(1,1)	2(1)	1,4(0,8)
Pt 6	7%	3,9(2,8)	--	--	0.9	--	3,5(5,9)
Pt 7	14%	0.2	--	--	1,2(0,3)	0.8	2,8(1,7)
Pt 8	7%	6,2(4,6)	--	--	0.4	0.3	0,7(1,3)
Pt 14b	14%	0,9(2,4)	--	--	0.4	--	1.4
Pt 6a	20%	1.8	--	--	--	--	--
Paratyphi B, var, Java	0%	0.2	0.3	--	23,6(40,1)	34,6(55,3)	--(3,4)
Infantis	16%	1.4	1.0	--	8,1(29,8)	7,6(18,1)	5,7(34)
Dublin	0%	0.4	0.3	44.9	--	--	--
Senftenberg	29%	0.2	0.5	--	9,5(3,3)	2.8	25,5(13,9)
Derby	7%	0.6	13,4(7,9)	2.1	1,8(0,5)	2,3(1)	0,7(0)
Virchow	34%	0.8	--	1.1	5.8	2.8	12.1
Livingstone	6%	0.4	1.5	--	1.1	1.1	--
Mbandaka	8%	0.6	0.3	1.6	4,9(2,2)	4,5(1,8)	2.1
Anatum	30%	0.4	7.7	8.0	0.5	0.6	--
Agona	23%	0.5	0.5	--	2,8(1,5)	1.7	5.0
Brandenburg	0%	1.2	2.3	2.7	0.5	0.3	1.4
Goldcoast	0%	0.8	0,3(6,1)	1,1(0)	0.2	--	0.7
Weltevreden	50%	0.1	4,4(0)	5,9(0)	0.4	--	--
Gallinarum	--	--	--	--	2,1(0,3)	0.3	5,7(1,3)
Hadar	13%	1.0	--	--	1,9(0,4)	2,3(0,6)	1,4(0,4)
Montevideo	18%	0.4	0.0	0.5	1.1	1.1	0.7
Panama	11%	0.5	4,6(1,1)	--	0.2	0.3	--
Lexington	--	--	0.3	6,4(0)	--	--	--
Rissen	--	--	4,1(0,4)	0.5	0.5	--	1.4
London	5%	1.0	0.5	0.9	1.4	--	0.3
Blockley	0%	0.1	--	--	2,8(0,1)	3,7(0)	--
Indiana	0%	0.2	--	--	1.1	1.1	0.7
Albany	33%	0,1(0)	--	--	2,1(0)	2,8(0)	1,4(0)

[2] Source: Report on trends and sources of zoonotic agents in the EU, 2004, The Netherlands

		Humans	Pigs	Cattle	Poultry	Broilers	Layers
Total number sent to RIVM		**1626**	**388**	**187**	**568**	**355**	**141**
Sero/phage type	**% Travel**	**% of the total sent to RIVM in 2004 (2003 data between brackets)**					
Corvallis	13%	0,4(0,1)	--	--	1,6(0)	2,5(0)	0.0
Heidelberg	4%	0.8	--	--	--(0,8)	--(0,6)	--(1,3)
Newport	21%	0.8	1,5(0)	0.5	0.2	0.3	--
Thompson	--	--	--	1,1(0)	0.5	0.3	1,4(0)
Table 1 continued							
(Para)Typhi (A B C)	35%	1.9	--	--	--	--	--
Kentucky	50%	1,3(0,3)	--	0.5	0.2	0.3	--
Give	0%	1(0,1)	--	--	--	--	--
Other serovars		7.2	8.6	7.2	12.1	16.9	9.0

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.

Table 10. MIC distribution (in %) for all salmonella's (N = 2195) tested for antibiotic susceptibility in 2004.

Total 2004	MIC distribution (µg/ml)															R%
	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	
Amoxicillin				21.2	62.0	1.7						15.1				15.2
Cefotaxim		90.3	8.3	1.0	0.05				0.1	0.2						0.3
Imipenem		84.1	14.9	0.9												0.0
Gentamicin			32.1	56.8	10.0	0.7			0.2	0.1	0.1					0.5
Neomycin					90.2	8.2	0.6			0.1	0.1	0.5	0.3			1.0
Tetracycline				0.1	17.6	62.5	3.8	0.3	0.3	4.9	3.1	7.3				15.6
Sulphameth.								32.5	48.7	2.4	0.05			0.1	16.2	16.3
Trimethoprim				85.6	5.7	1.0	0.1		0.1			7.6				7.7
Ciprofloxacin	91.4	2.9	3.6	1.5	0.4			0.23	0.05							0.3
Nalidixic Acid						3.7	79.1	8.3	0.8	0.1		0.6	7.4			8.2
Chloramphenicol							6.5	79.7	6.6	0.2	0.1	0.4	6.5			7.2
Florfenicol						0.7	73.2	19.0	1.1	4.7	0.7	0.4	0.14			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. The vertical bars indicate the breakpoints.

Table 10 presents MIC-distributions and resistance percentages of all salmonella's tested for susceptibility in 2004. Highest levels of resistance were observed for sulphamethoxazole, tetracycline and amoxicillin and to a lesser extend nalidixic acid, trimethoprim and chloramphenicol.
Seven cefotaxime resistant, ESBL suspected strains were found, which was less than in 2003 (n = 13). These isolates belonged to the following serovars: 1 *S.* Braenderup, 2 *S.* Paratyphi B var. Java, and 3 *S.* Infantis from poultry and 1 *S.* Enteritidis Pt 14b isolated from a human patient. Except the two *S.* Java strains they were susceptible to the other antibiotics included in the test. It is the third consecutive year that ESBL-positive *S.* Java strains were detected.
The *S.* Braenderup and *S.* Infantis were also resistant to clavulanic acid; *S.* Infantis was also resistant to cefoxitin and is therefore *AmpC*-suspected. The two *S.* Java strains showed characteristics of *CTX-M* (cefotaxime R, ceftazidime I), the other strains showed a typical ESBL-phenotype (cefotaxime and ceftazidime R, a synergistic effect of the antibiotics combined with clavulanic acid and cefoxitin S).
The beta-lactamase genes in these salmonella's and in those detected in *E. coli* will be typed

molecularly in co-operation with dr. Ernesto Liebana, Veterinary Laboratories Agency, Weighbridge, UK. This data will be reported separately.

Eleven gentamicin resistant strains, and twenty two neomycin resistant strains were found, the majority isolated from human patients. Four of the gentamicin resistant strains were also high level ciprofloxacin resistant (*S.* Kentucky).

Six ciprofloxacin resistant S. Kentucky strains (MIC ≥ 8 µg/ml) were isolated from human patients (in 2002 and 2003 also ciprofloxacin resistant *S.* Kentucky's were isolated from human patients). These strains are related to travel to Egypt. Obviously clonal spread of a fluoroquinolone resistant S. Kentucky in Egypt resulted in human salmonella infections in tourists.

One hundred seventy nine (in 2002 168 and in 2003 271) nalidixic acid resistant strains were found. These strains all showed reduced susceptibility to ciprofloxacin (MIC ≥ 0,125 µg/ml). In 2004 4 *S.* Corvallis strains 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). The genetic basis of this phenotype is yet unknown.

Sixteen fully susceptible *S.* Newport strains were found, one isolated from poultry, thirteen from human patients and two from soy products.

Table 11. Resistance percentages of the ten most prevalent *Salmonella* serovars isolated in The Netherlands in 2004.

	Enteritidis (623)	**Typhimurium (460)**	**Dublin (87)**	**Senftenberg (75)**	**Mbandaka (56)**	**Infantis (53)**	**Java (36)**	**Derby (28)**	**Livingstone (26)**	**Virchow (25)**	**Anatum (20)**
Amoxicillin	3.0	47.6	1.1	2.7	8.9	13.2	72.2	17.9	0	20.0	5.0
Cefotaxime	0.2	0	0	0	0	5.7	5.6	0	0	0	0
Imipenem	0	0	0	0	0	0	0	0	0	0	0
Gentamicin	0	0.2	0	0	0	0	0	0	3.8	0	0
Neomycin	0.2	1.1	0	0	0	1.9	2.8	0	0	0	0
Tetracycline	0.3	55.4	1.1	1.3	7.1	3.8	5.6	35.7	0	12.0	15.0
Sulphamethox.	0.8	50.7	10.3	4.0	12.5	7.5	72.2	42.9	19.2	20.0	5.0
Trimethoprim	0.3	15.4	1.1	4.0	7.1	5.7	100	42.9	19.2	20.0	5.0
Ciprofloxacin	0	0	0	0	0	0	0	0	0	0	0
Nalidixic acid	12.2	3.0	3.4	0	0	5.7	36.1	7.1	0	80.0	0
Chloramphenicol	0.2	28.0	9.2	1.3	0	1.9	0	7.1	0	0	0
Florfenicol	0	27.6	0	0	0	0	0	3.6	0	0	0
% fully sens	85%	35%	86%	96%	84%	81%	0%	43%	81%	12%	85%
%R to 1 an	14%	14%	3%	0%	5%	8%	17%	11%	0%	64%	10%
%R to 2 ant	1%	4%	9%	1%	2%	6%	11%	14%	15%	4%	0%
%R to 3 ant	0%	14%	0%	1%	9%	2%	39%	21%	4%	8%	0%
%R to 4 ant	0%	4%	1%	0%	0%	0%	28%	7%	0%	4%	5%
%R to >4 ant	0%	28%	0%	1%	0%	4%	6%	4%	0%	8%	0%

In table 11 resistance percentages are presented for the most prevalent serovars isolated in The Netherlands in 2004. The highest resistance levels are observed in *S.* Typhimurium, *S.* Java and *S.* Derby, the serovars also harbouring the highest percentages of multiple resistant isolates.

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 low (Tables 9 and 15). The difference in resistance profile of strains from human infections and Dutch poultry indicates that other sources of infection exist. In 2004 from human infections 75 nalidixic acid-resistant strains were isolated, predominantly Pt1 (46%) and to a lesser extend Pt8 (13%) and Pts 4 and 6a (11%). In Dutch poultry no nalidixic acid-resistant strains were found.

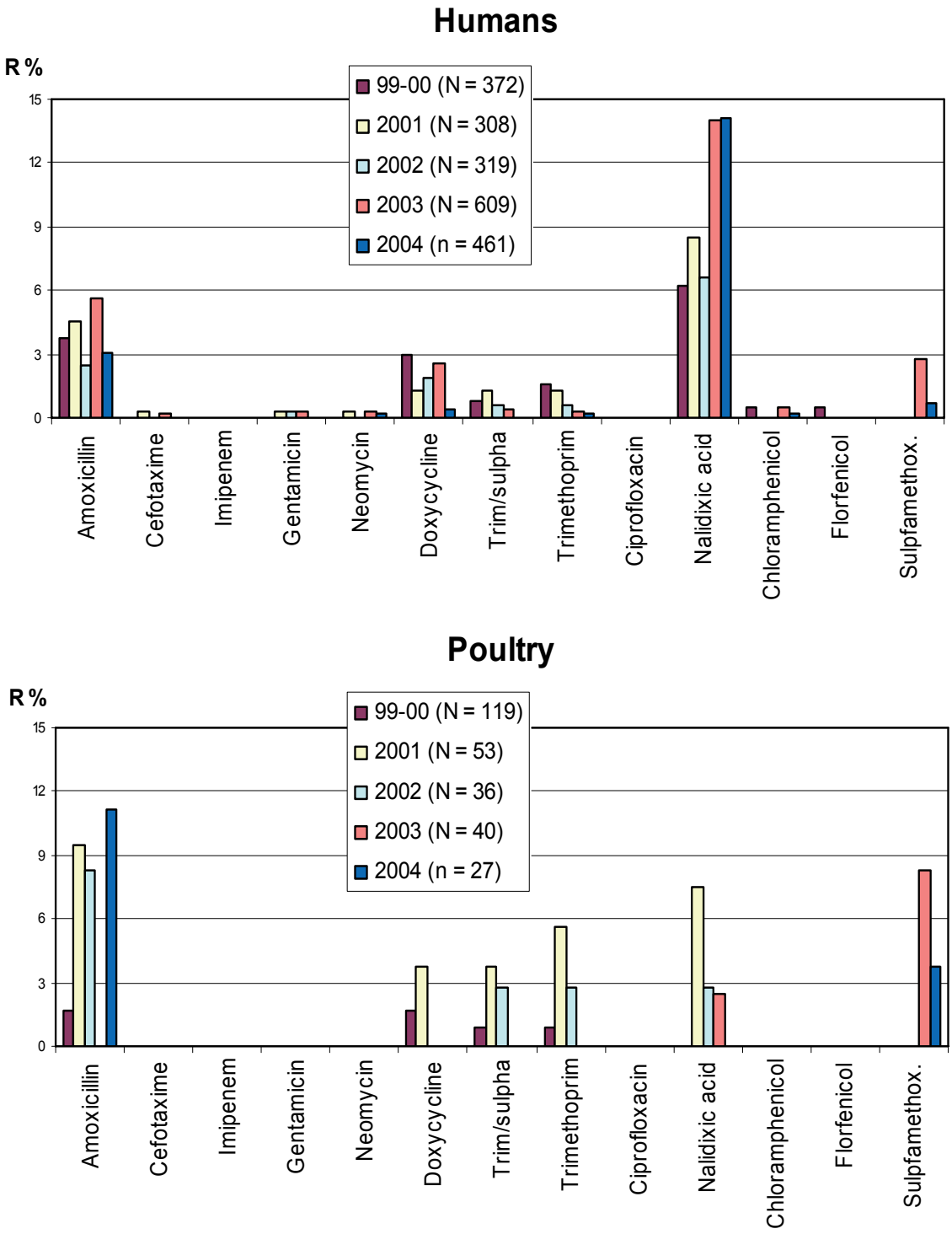
Table 12. Resistance percentages of *S.* Enteritidis and phagetypes 4, 21, 8, 1, 6, 6a, 16a and 14b isolated from different sources in 2004.

	***S.* Enteritidis**		**Phage types**						
	Human (461)	**Dutch poultry (27)**	**pt4 (205)**	**pt21 (125)**	**pt8 (77)**	**pt1 (71)**	**pt6 (54)**	**pt6a (22)**	**pt14b (15)**
Amoxicillin	3.0	11.1	1.5	0	0	2.8	0	40.9	6.7
Cefotaxime	0.2	0	0	0	0	0	0	0	6.7
Imipenem	0	0	0	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	0	0	0	0
Neomycin	0.2	0	0	0	0	0	0	4.5	0
Tetracycline	0.4	0	0	0	0	1.4	1.9	0	0
Sulphamethox	0.7	3.7	0.5	0	0	1.4	1.9	9.1	0
Trimethoprim	0.2	0	0	0	0	0	1.9	4.5	0
Ciprofloxacin	0	0	0	0	0	0	0	0	0
Nalidixic acid	14.1	0	4.4	1.6	13.0	50.7	5.6	36.4	6.7
Chloramphenicol	0.2	0	0	0	0	0	0	4.5	0
Florfenicol	0	0	0	0	0	0	0	0	0
% fully sens	85%	88%	94%	98%	87%	48%	94%	27%	91%
%R to 1 ant	13%	12%	6%	2%	13%	49%	4%	55%	5%
%R to 2 ant	1%	0%	0%	0%	0%	1%	0%	9%	5%
%R to 3 ant	1%	0%	0%	0%	0%	1%	0%	9%	0%
%R to 4 ant	0%	0%	0%	0%	0%	0%	2%	0%	0%
%R to >4 ant	0%	0%	0%	0%	0%	0%	0%	0%	0%

Multiple resistance is not very common in *S.* Enteritidis. Incidentally multiple resistant strains were observed in PT1, Pt6, Pt6a and Pt14b.
Trends in resistance are limited to nalidixic acid resistance in human isolates (Fig. 10). The observed increase in 2003 related to increase of imported eggs due to the influenza outbreak, has not been followed by a decrease in 2004.

It can be concluded that nalidixic acid resistant strains of *S.* Enteritidis isolated from humans either originate from imported eggs or from travel related infections. Therapy of humans may have contributed as well.

Figure 10. Trends in resistance percentages of *S.* Enteritidis isolated from humans and poultry (predominantly from Dutch layers and reproduction animals, whilst poultry meat is of mixed Dutch and imported origin) from 1999 - 2004.



S. Typhimurium

Resistance percentages of *S.* Typhimurium in 2004 were strongly determined by the presence of multi drug resistant phage types DT104, Ft 510, Ft 507 and Ft 508, being among he predominant phage types of *S.* Typhimurium, both in food-animals and in humans (table 13).

In 2003 thirteen nalidixic acid resistant *S.* Typhimurium isolates were found, in 2004 fourteen. Seven were DT104, three were Ft 507, two Ft 12, and one Ft 508 and 510, respectively. Thirteen of these strains were isolated from human patients; one DT104 was isolated from pig faeces. All nalidixic acid resistant isolates demonstrated reduced susceptibility to ciprofloxacin but were not high-level ciprofloxacin resistant.

Resistance levels and multiple resistances were substantially higher in *S.* Typhimurium than in *S.* Enteritidis (table 5, Fig. 11). Approximately 50% of the strains were resistant to three or more antibiotic classes in poultry and pig isolates. In humans isolates this was 33% and cattle the level of multiple resistance was highest (61%).

Trends in resistance in *S.* Typhimurium are difficult to determine in all sources (Fig. 12) because of the influence of the presence of multiple resistant clones and the relatively small number of isolates from cattle and poultry. Specifically when the total numbers of strains per year are relatively small the variability in the resistance percentages is high (eg. in poultry). In 2004 in pigs an increase was observed in resistance to amoxicillin, tetracycline, sulphamethoxazole and the fenicols, chloramphenicol and florfenicol, as a result of the clear increase in proportion of DT104 isolated from pigs, 35% in 2004 compared to 19% in 2003.
In human isolates the level of nalidixic acid resistance (3.9%) was higher than in animal isolates and cannot be explained by the proportion of DT104 present. Therefore also in *S.* Typhimurium either imported animal products, travel or human therapy may have contributed to nalidixic acid resistance..

Table 13. Resistance percentages of S. Typhimurium and phage types DT104, Ft 507, FT510, Ft 508 and Ft401 isolated from different sources in 2004.

	Human (334)	Pigs (77)	Cattle (13)	Poultry (9)	DT104 (129)	ft507 (121)	ft510 (32)	ft508 (31)	ft401 (20)
Amoxicillin	49.1	51.9	53.8	44.4	89.9	47.9	25.0	48.4	70.0
Cefotaxime	0	0	0	0	0	0	0	0	0
Imipenem	0	0	0	0	0	0	0	0	0
Gentamicin	0.3	0	0	0	0	0.8	0	0	0
Neomycin	1.2	0	0	0	0	3.3	3.1	0	0
Tetracycline	53.6	72.7	61.5	33.3	90.7	51.2	68.8	45.2	90.0
Sulphamethox.	48.8	67.5	69.2	44.4	92.2	50.4	31.3	45.2	65.0
Trimethoprim	12.3	29.9	15.4	22.2	14.7	27.3	15.6	6.5	5.0
Ciprofloxacin	0	0	0	0	0	0	0	0	0
Nalidixic acid	3.9	1.3	0	0	5.4	2.5	3.1	3.2	0
Chloramphenicol	28.4	32.5	38.5	22.2	85.3	3.3	12.5	29.0	5.0
Florfenicol	28.1	31.2	38.5	22.2	85.3	3.3	9.4	29.0	5.0
% fully sens	55%	37%	31%	40%	3%	32%	28%	48%	10%
%R to 1 ant	10%	13%	8%	10%	7%	17%	41%	3%	20%
%R to 2 ant	3%	4%	0%	0%	3%	5%	0%	3%	5%
%R to 3 ant	9%	12%	15%	20%	1%	32%	13%	16%	60%
%R to 4 ant	3%	4%	8%	0%	1%	9%	6%	0%	0%
%R to >4 ant	21%	29%	38%	30%	85%	6%	13%	29%	5%
DT104: %	28%	35%	38%	22%	-	-	-	-	-

Figure 11. Percentages of S. Typhimurium strains fully susceptible, resistant to one, two, three, four and more than four antibiotics in humans, pigs, cattle and poultry in The Netherlands in 2004.

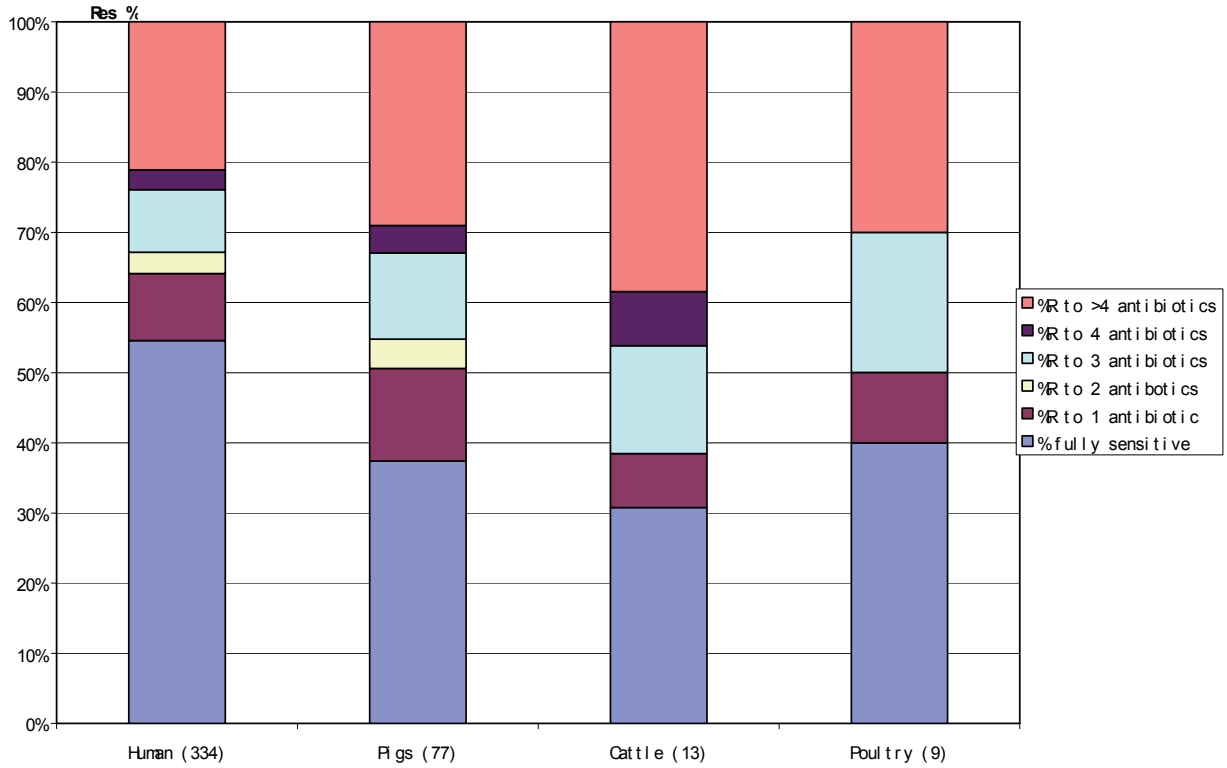
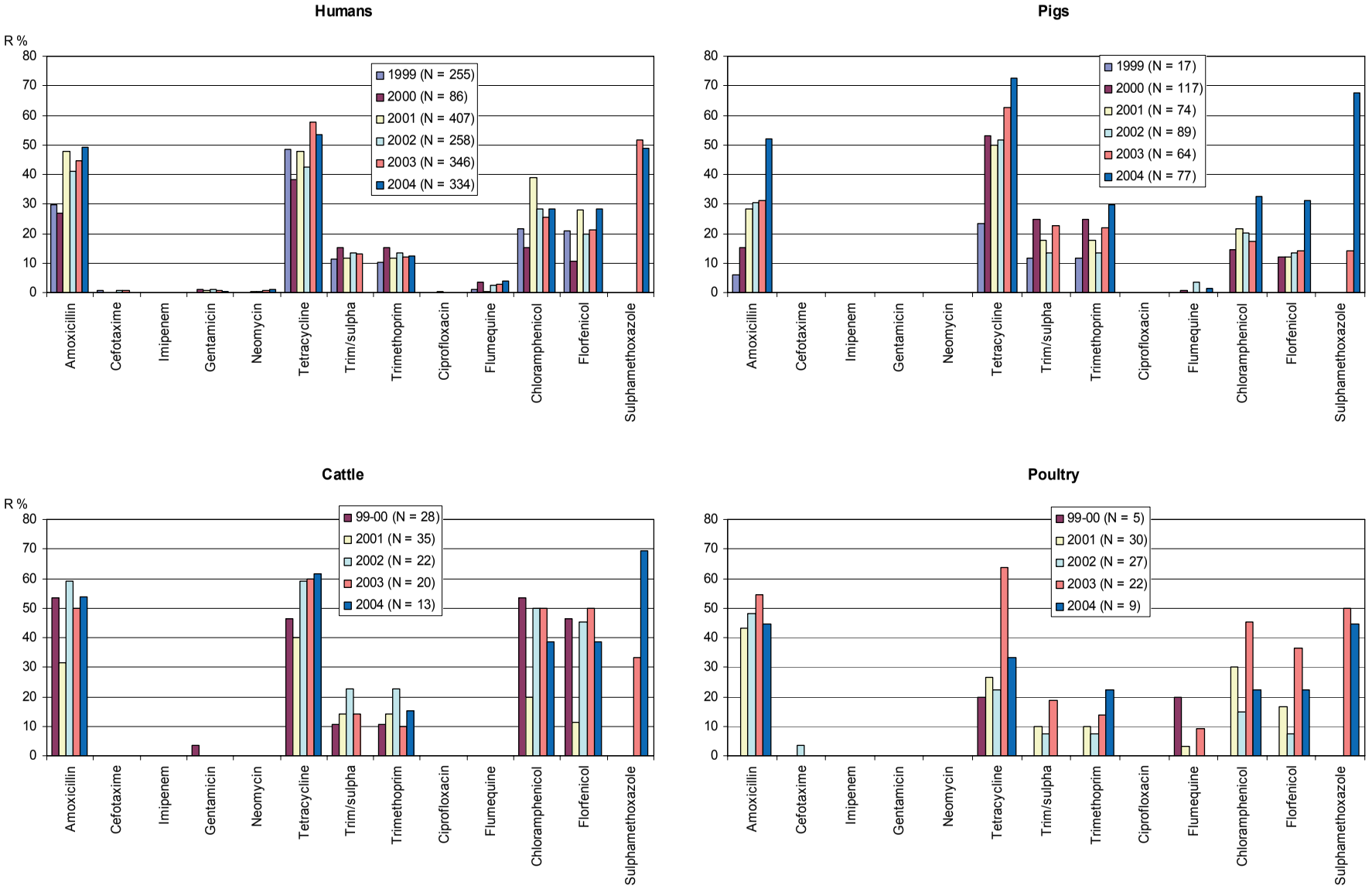


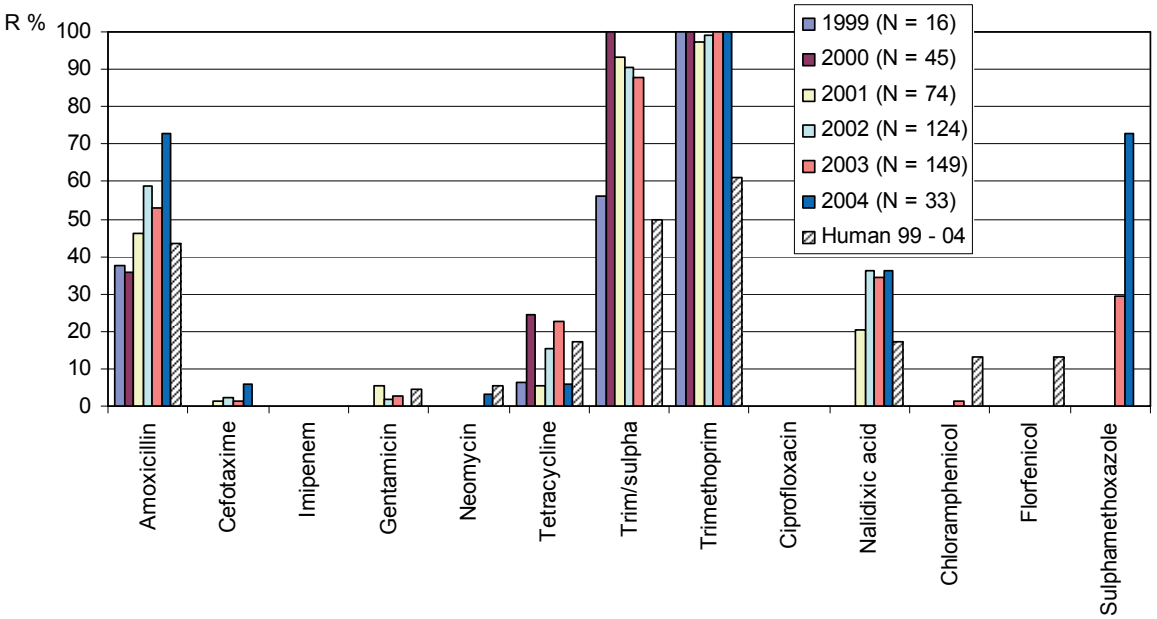
Figure 12. Trends in resistance percentages of *S.* Typhimurium isolated from humans and food-animals from 1999 - 2004



S. Paratyphi B var. Java

S. Java is the predominant serovar isolated from broilers since 1998, however the proportion of of *S.* Java of all Salmonella's that were sent in for typing in broilers decreased from 55.3% in 2003 to 34.6% in 2004. At retail however (statistical sampling, table 15) no decrease is indicated. In 2004 three strains with a resistance profile typical of the clone were isolated from Dutch human patients. From broilers and broiler products 33 strains were isolated, all harbouring the phenotype typical for the clone. Nalidixic acid resistance in *S.* Java isolated from poultry has remained stable in 2004 (Fig. 13). No ciprofloxacin resistant strains were found. Resistance to amoxicillin and sulphamethoxazole shows a tendency to increase in 2004. Also cefotaxime resistance (ESBL-producers) shows a tendency to increase. Third-generation cephalosporins are not used in broilers, therefore the use of other beta-lactam antibiotics or even other classes of antibiotics may select for beta-lactamases which are often located on integrons harbouring more resistance genes.

Figure 13. Trends in resistance percentages of *S.* Paratyphi B var. Java isolated from poultry from 1999 – 2004 and humans (blue bars indicate all humans isolates from 1999 – 2004 (N = 23))

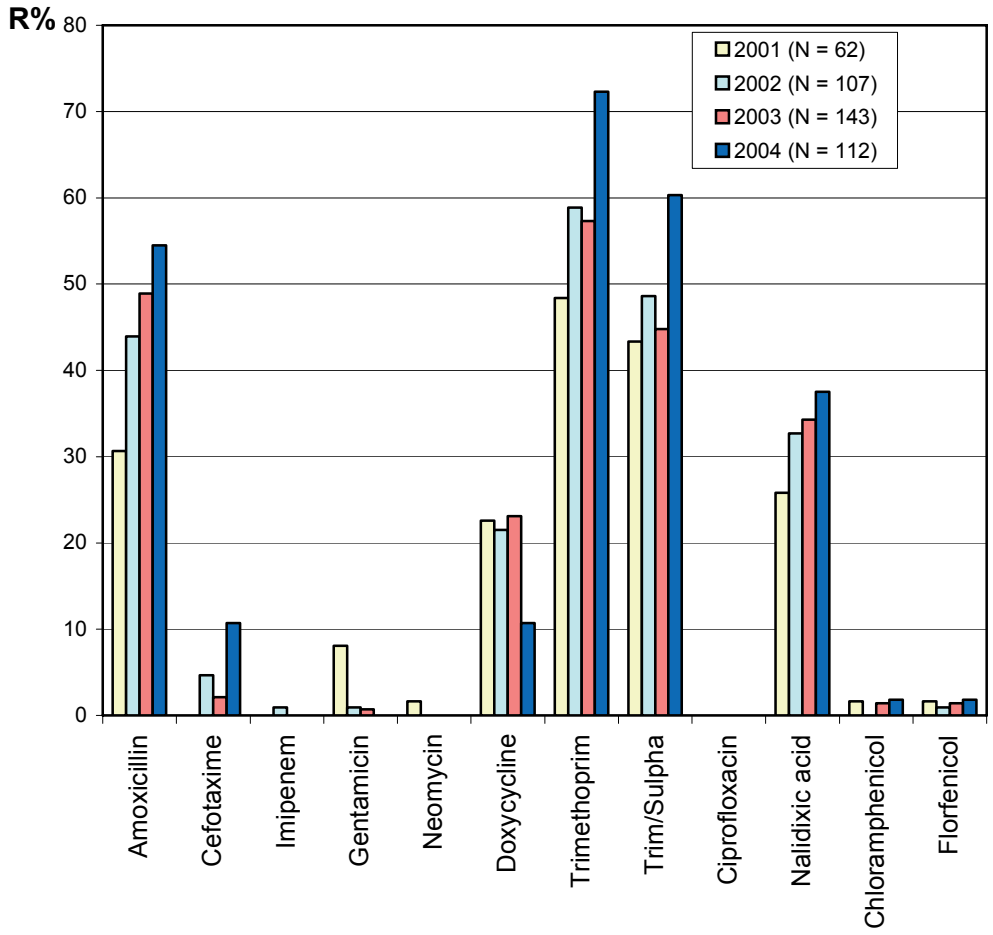


Salmonella spp. in raw meat products of food-animals

Table 14. Resistance % of *Salmonella* spp. isolated from raw meat from poultry, beef and pork products in 2004

	Poultry N = 112	Beef N = 4	Pork N = 7
Amoxicillin	54.5	75	0
Cefotaxime	10.7	0	0
Imipenem	0	0	0
Gentamicin	0	0	0
Neomycin	0	0	0
Doxycycline	10.7	50	28.6
Trim/suplha	60.3	25	0
Trimethoprim	72.3	25	0
Sulphamethoxazole	45.5	-	-
Ciprofloxacin	0	0	0
Nalidixic acid	37.5	0	0
Chloramphenicol	1.8	25	0
Florfenicol	1.8	0	0

Figure 14. Trends in resistance % of *Salmonella* spp. isolated from chicken products in the Netherlands from 2001 – 2004.



In general the resistance levels of Salmonella's isolated from raw meat products are highest in poultry products compared to beef and pork, although the number of isolates tested from beef and pork are too small to draw firm conclusions (table 14). The observed resistance patterns and trends in the chicken isolates are strongly determined by the large contribution of *S.* Java (table 15). In beef and pork resistance is limited to older drug classes, while only in poultry products resistance to third-generation cefalosporins (cefotaxime), gentamicin and the quinolones occurs. Similar as observed in strains isolated from poultry faeces, an increase in amoxicillin and cefotaxime resistance was observed. Resistance trends are only presented for poultry products because in beef and pork the numbers of isolates examined are too small to provide an accurate estimate (Fig. 14).

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**
sample size	1314	1077	859	1454	1578	1600	1510	1482
Salmonella spp. positive (%)	29.1	20.2	17.6	21	16.3	13.4	11.3	7.4
	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
Enteritidis	20.2	12.8	26.4	6.6	8.2	2.3	8.8	5.5
Hadar	10.1	6.1	4.5	3.3	4.2	0.9	1.8	-
Indiana	6.1	8.3	9.3	10.2	11.6	6.5	6.4	1.8
Infantis	9.2	5	3.6	6.6	7	7.9	11.7	-
Virchow	4.6	2.8	2.6	10.2	3.5	5.6	5.8	4.5
Typhimurium (DT104)	7.8	3.6(1.8)	1.3(0.7)	0.1(0.1)	7.4(7)	7.4(2.8)	5.8(5.3)	3.6
Other types	27	50	38.4	29.9	14.9	15.8	5.8	9.1

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

In table 16 resistance data are presented for salmonella's isolated from animal feeds and more incidental animal sources. A wide variety of serovars were isolated, *S.* Senftenberg, *S.* Agona, *S.* Mbandaka, *S.* Lexington were the most prevalent ones. The resistance percentages were much lower than those for the human and food-animal isolates and highest in *S.* Livingstone in different feed sources.

In Salmonella's isolated from turkeys, horses and ducks, more resistance was observed than in strains from pigeon or reptiles. Nalidixic acid resistance was highest in turkeys and ducks.

Table 16. The most prevalent serovars isolated from animals feeds and resistance percentages (R%) of isolates of *Salmonella* spp. per single and or compound feed type, in 2001 - 2003 combined and 2004. Moreover R% of Salmonella strains isolated from incidental animal sources over 2001 – 2004 are presented.

			Animal feed (or ground substance)								Animals				
Serovars tested from feed, 2001-2004			**Fish meal (42)**	**Animal meal (29)**	**Soy (feed, N=495)**	**Rapeseed (feed, N=228)**	**Single feed, other (187)**	**Composite feed (98)**	**Feed 2004, N=453**	**Feed 2001-2003, N=626**	**Turkey (25)**	**Horse (34)**	**Duck (10)**	**Pigeon (30)**	**Reptilian/Amfibian (69)**
Senftenberg	139	**Antibiotics**	**% Resistant 2001-2004**						**R %**	**R %**	**%-Resistant 2001-2004**				
Agona	136	Amoxicillin	0	1	2	3	3	1	0.4	1	28	21	30	13	1
Mbandaka	103	Cefotaxime	0	0	0	0	0	0	0	0	0	0	0	0	0
Lexington	94	Imipenem	0	0	0	0	0	0	0	0	0	0	0	0	0
Rissen	61	Gentamicin	0	0	0	0	0	0	0	0	4	0	0	0	0
Anatum	59	Neomycin	0	0	0	0	0	0	0	0	12	0	0	0	0
Livingstone	43	Tetracycline	0	1	14	4	10	8	0.7	5	28	24	10	13	3
Tennessee	40	Sulfamethox	0	0	2	1	0	0	0.7	0	4	0	0	3	0
Havana	39	Trimethoprim	0	1	3	1	1	2	0.7	1	4	21	0	0	0
Cubana	38	Ciprofloxacin	0	0	0	0	0	0	0	0	0	0	0	0	0
Kentucky	34	Nalidixic acid	0	1	0	0	2	2	0.2	1	20	3	20	0	0
Oranienburg	31	Chloramph.	0	1	2	3	4	2	0.7	1	0	18	10	13	1
Montevideo	25	Florfenicol	0	0	2	3	2	0	0.4	1	0	6	0	13	1
13 main serovars	842(78%)														
All serovars	1079														

Campylobacter spp.

Highlights
Highest resistance levels were observed in *C. coli* from pigs. Resistance to the quinolones was substantially higher in poultry, reflecting the use pattern of this antimicrobials class in these animals. Resistance to erythromycin was only present in *C. coli* and highest in strains from pigs.
Also the prevalence of multiple resistant strains was highest in pigs compared to poultry. A tendency to increase in resistance can be observed in poultry for amoxicillin and doxycycline. Resistance to nalidixic acid and ciprofloxacin is stable.
In domestically acquired human infections with *C. jejuni* up to 2% of the isolates were reported resistant to erythromycin. Because in *C. jejuni* strains isolated from Dutch poultry until 2005 not one erythromycin resistant strain has been detected, human infections with *C. jejuni* strains resistant to erythromycin are most likely travel related, caused by consumption of contaminated imported products or due to human therapy.

Table 17. MIC distribution (in %) for all *Campylobacter* spp. isolated from broilers and slaughter pigs (N = 277) in The Netherlands in 2004

Campylobacter* spp. *2004	**MIC % distribution (µg/ml)**														
	0.125	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**R%**
Amoxicillin		0.7	4.0	11.6	17.8	25.4	13.4	0.7	6.5	19.9					26.4
Gentamicin		65.0	33.9	0.7	0.4										0
Neomycin			24.9	67.5	4.3	0.7		1.4	0.7	0.4					2.5
Streptomycin				23.1	10.1	3.6	0.4	5.1	37.5	10.8	2.5	7			62.8
Doxycycline	13	6.9	2.9	1.1	1.8	6.5	23.1	31.0	13.7						67.9
Trim/suplha		2.5	10.8	15.5	11.6	7.2	4.3	22.7	23.5	1.8					48
Sulphamethoxazole							9.7	16.6	8.3	11.6	4	12.3	33.2	4.3	37.5
Ciprofloxacin	43.3	31.8	6.9	0.7		0.4	3.2	8.3	5.4						17.3
Nalidixic acid				1.4	14.8	35.4	28.5	2.2		1.1	12.3	4.3			17.7
Erythromycin			2.5	2.9	16.6	32.5	32.1	5.4	0.7	0.7	6.5				13.4
Metronidazole			7.2	28.9	13	6.1	6.5	10.1	14.1	9.4	4.7				44.8
Chloramphenicol					19.9	46.2	28.2	5.4	0.4						0.4

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 campylobacters isolated from broilers and slaughter pigs in 2004. In table 10 these resistance percentages are presented separately for both animal and *Campylobacter* species and for both species isolated from poultry raw meat products. In Figure 15 the percentages of multiple resistance is presented for each animal and *Campylobacter* species, and the trends in resistance from 1999 – 2004 are presented in Figure 16.

The MIC-distributions are bimodal for most antibiotics and although, internationally accepted interpretive criteria are lacking, for most antibiotics the breakpoints used distinguishes resistant from susceptible populations (table 17).
Highest resistance percentages can be observed for streptomycin, doxycycline, metronidazole and (potentiated) sulphonamides. Resistance to the quinolones, erythromycin and amoxicillin are substantial. However differences in level of resistance exist both between *C. jejuni* and *C. coli*, and between pigs and broilers. Table 18 shows that except for amoxicillin, *C. coli* from poultry shows higher resistance levels than *C. jejuni* from poultry, as a result of the differences in species-specific capacity to become resistant. Moreover, resistance percentages for strains isolated from faeces or meat products are very similar. Highest resistance levels are observed in *C. coli* from pigs. Resistance to the

quinolones is substantially higher in poultry, reflecting the use pattern of this antimicrobials class in these animals. Resistance to erythromycin is only present in *C. coli* and highest in strains from pigs. In Campylobacter multiple resistance is highest in pigs compared to poultry. Resistance to three or more antibiotic classes in *C. coli* from pigs is present in 54% of the strains, in the same species from poultry in 38% of the strains and in *C. jejuni* in 40% of the strains (Fig. 15).

A tendency to increase in resistance can be observed in poultry for amoxicillin and doxycycline. Resistance to nalidixic acid and ciprofloxacin is stable (Fig. 16).

Table 18. Resistance percentages of *C. jejuni* and *C. coli* isolated from broilers and slaughter pigs in 2004

	Broilers *C. jejuni* (N = 57)	**Poultry products *C. jejuni* (N = 104)**	**Broilers *C. coli* (N = 21)**	**Poultry products *C. coli* (N = 55)**	**Pigs *C. coli* (N = 199)**
Amoxicillin	50.0	22.1	4.8	16.4	22.2
Gentamicin	0	0	0	1.8	0
Neomycin	3.5	1.0	4.8	5.5	0
Streptomycin	0	1.0	42.9	20.0	83.3
Doxycycline	45.6	24.0	57.1	61.8	75.3
Trim/suplha	7.0	4.8	33.3	18.2	61.6
Sulphamethoxazole	5.3	4.8	28.6	16.4	48.0
Ciprofloxacin	40.4	39.4	52.4	69.1	7.1
Nalidixic acid	40.4	39.4	52.4	70.9	7.6
Erythromycin	0	0	4.8	7.3	18.2
Metronidazole	57.9	66.3	42.9	50.9	40.9
Chlooramphenicol	1.8	0	0	0	0
% fully Sensitive	19%	13%	5%	5%	5%
% R to 1 antibiotic	18%	45%	24%	16%	15%
% R to 2 antibiotics	23%	21%	33%	31%	26%
% R to 3 antibiotics	26%	11%	19%	31%	25%
% R to 4 antibiotics	7%	7%	14%	7%	16%
% R to > 4 antibiotics	7%	3%	5%	9%	13%

Figure 15. Percentages of *Campylobacter* strains isolated from faecal samples fully susceptible, resistant to one, two, three, four and more than four antibiotics in pigs and poultry in The

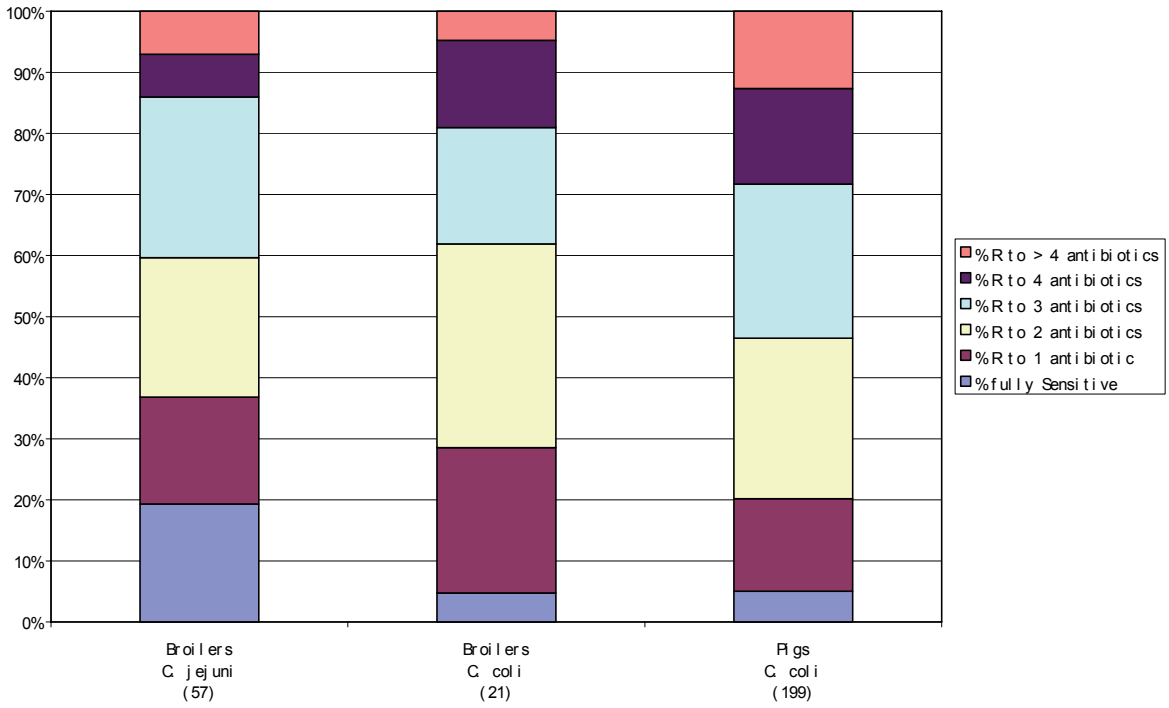


Figure 16. Trends in resistance percentages of *C. coli* isolated from slaughter pigs and broilers (grey striped bars), and *C. jejuni* isolated from broilers from 1999 - 2004

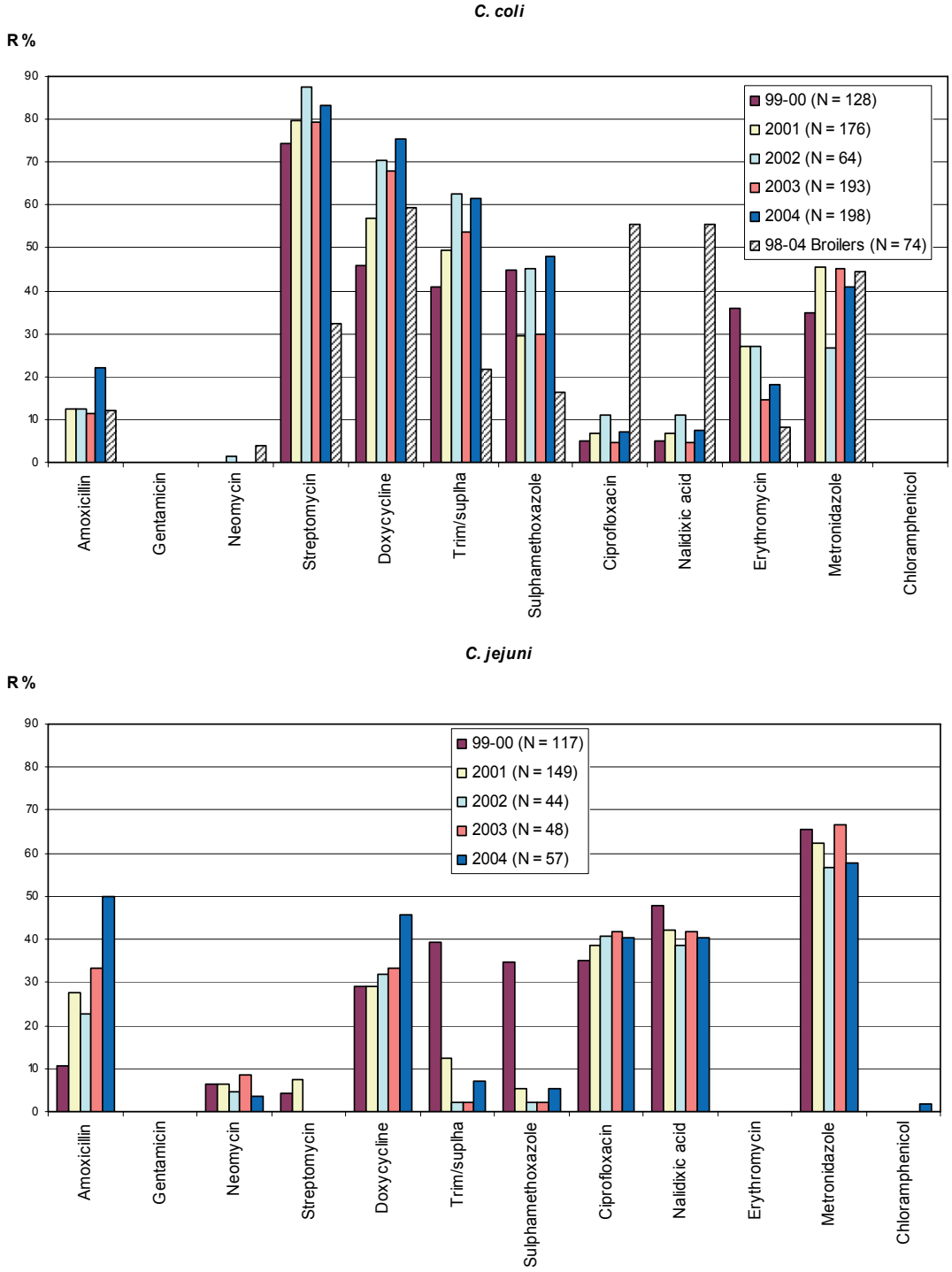


Figure 17 shows that in human *Campylobacter* spp. resistance to fluoroquinolones (data are based on disk diffusion tests for norfloxacin, ofloxacin and ciprofloxacin) slowly increased in the last decade, but remained stable around 31% since 2000. In 2000 both resistance to fluoroquinolones and tetracyclines increased suddenly approximately 10%. A biological explanation for this phenomenon does not exist. Resistance to macrolides remained stable at a very low level.

Disk diffusion is not advocated by CLSI for susceptibility testing of campylobacters and guidance on interpretive criteria is lacking, therefore these data have to be interpreted with care.

Figure 17. Trends in resistance % of *Campylobacter* spp. isolated from humans isolated between 1993 and 2003 at the regional Public Health Laboratories (PHLs) of Arnhem and Heerlen covering 990.000 inhabitants. The dotted line represent data from the national surveillance in 2002 - 2004; annually the average number of strains tested was approximately 2400, ranging from 1900 – 2900.

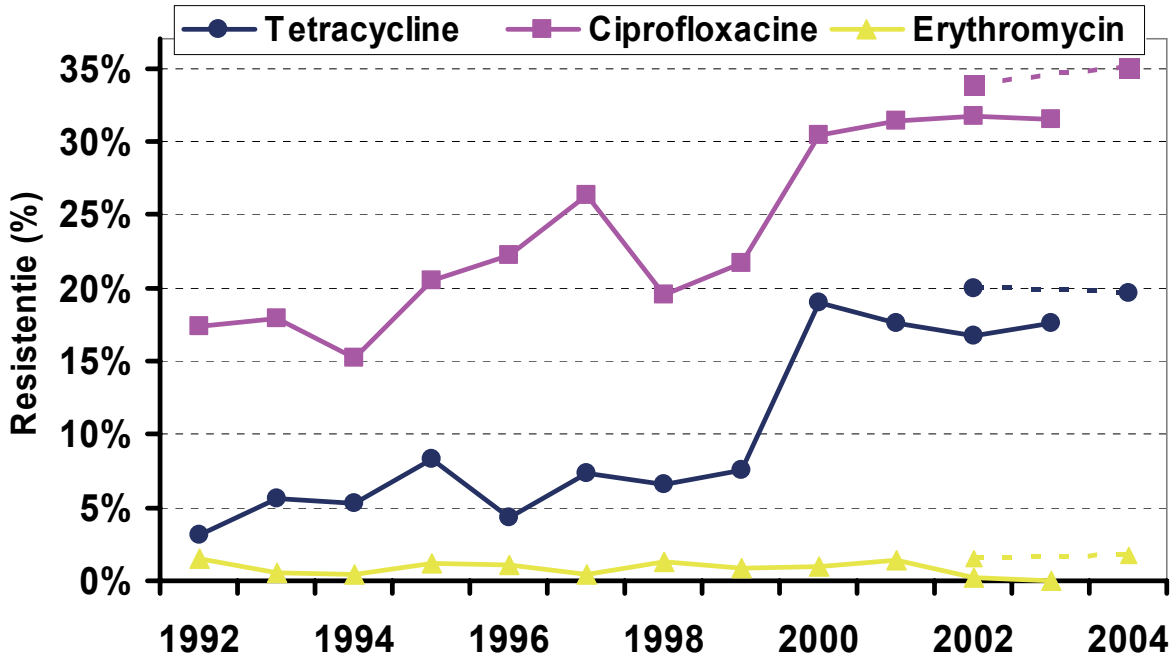


Table 19A. Domestically acquired and travel related resistance in *C. jejuni* and *C. coli* isolated from humans in 2004 from all 16 PHLs covering > 50% of the Dutch population and the resistance percentages in strains isolated by a general practitioner and a specialist.

2004 data	Domestically acquired				Travel related				All Campylobacter species			
	C. jejuni		C. coli		C. jejuni		C. coli		Gen. Pract.		Specialist	
	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	2092	32.6	97	33.0	188	54.3	14	64.3	2055	34.8	570	35.8
Tetracycline	1499	18.9	85	18.8	118	21.2	13	15.4	1530	19.3	372	21.2
Erythromycin	1816	1.7	90	2.2	163	1.2	14	--	1811	1.6	487	2.1

Table 19B. Effect of degree of urbanisation and rural source of human *C. jejuni* isolates on resistance percentages

***C. jejuni*, not travel-related, 2004 isolates**		
Degree of urbanisation	**Urban**	**Rural**
Fluoroquinolone (N)	786	421
R%	34.8	27.6
Tetracycline (N)	598	277
R%	23.2	13
Erythromycin (N)	707	359
R%	2.1	0.8

Table 19A shows that in travel-related infections fluoroquinolone resistance occurred more frequently than in isolates from domestically acquired infections, for tetracycline and erythromycin this difference was not observed. No difference in resistance levels existed between campylobacters isolated in general practice compared to those after submission to a hospital (specialist).
In domestically acquired strains of C. *jejuni* the resistance percentages are higher than in rural strains from areas (table 19B). This indicates that different sources of infection exist.

In *C. jejuni* strains isolated from Dutch poultry until 2005 not one erythromycin resistant strain has been detected. Therefore human infections with *C. jejuni* strains resistant to erythromycin (table 19A and 19B) 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 2004 78 strains of *E. coli* O157 were sent to RIVM for typing purposes or isolated from specimens taken from human faeces (37), veal calves and dairy cattle (41) in an attempt to trace a human clinical infection.

Highlights
The resistance levels for *E. coli* O157 were low and were slightly higher in cattle isolates compared to those from human sources. Resistance was limited to four older classes of antibiotics: amoxicillin, doxycycline, trimethoprim and sulphamethoxazole. Trends in resistance cannot be observed.

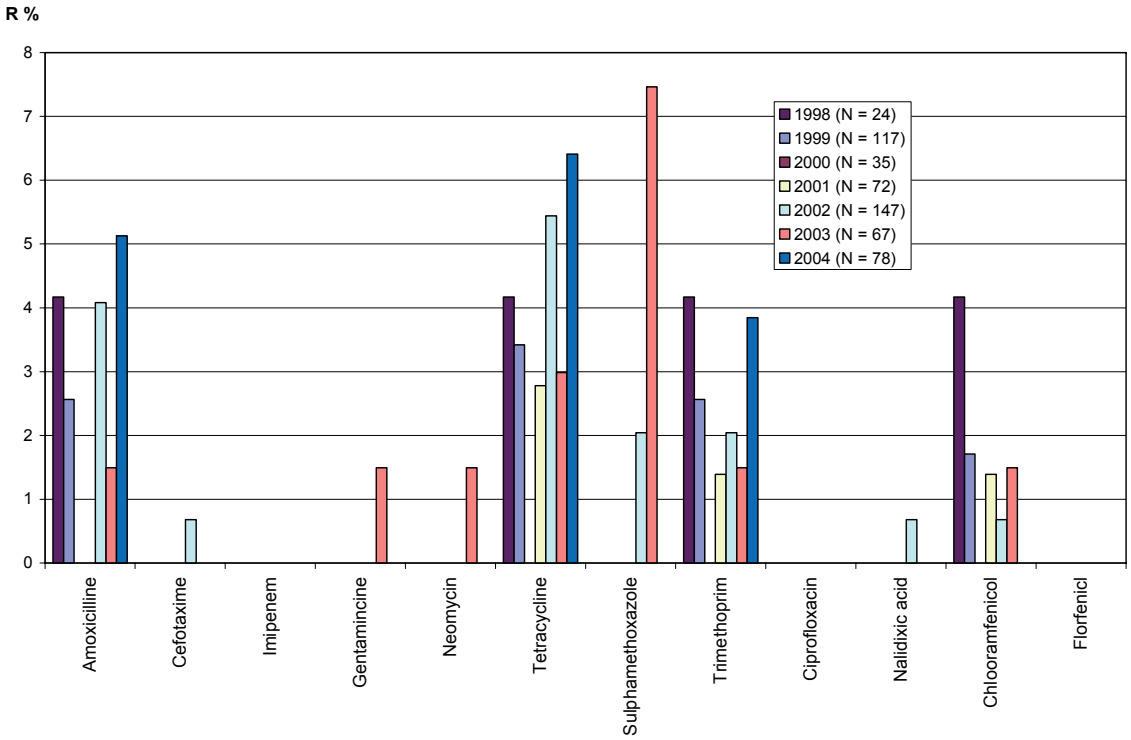
Table 20. MIC distribution (in %) for *E. coli* O157 isolated in The Netherlands in 2004 from human (N = 37) and cattle faeces (N = 41)

Humans (37)	MIC % distribution (µg/ml)																R%
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	
Amoxicillin									94.6					5.7				5.7
Cefotaxim				97.3	2.7													0
Imipenem				91.9	8.1													0
Gentamicin					8.1	70.3	16.2	5.4										0
Neomycin							89.2	8.1	2.7									0
Doxycycline							2.7	91.9	2.7					2.7				2.7
Sulphamethox										97.3							2.7	2.7
Trimethoprim						97.3								2.7				2.7
Ciprofloxacin			100															0
Nalidixic acid								13.5	86.5									0
Chloramphenicol										70.3	29.7							0
Florfenicol									2.7	97.3								0

Cattle (41)	MIC % distribution (µg/ml)																R%
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	
Amoxicillin								2.4	90.2	2.4				4.9				4.9
Cefotaxim				97.6		2.4												0
Imipenem				90.2	7.3	2.4												0
Gentamicin					19.5	70.7	4.9	4.9										0
Neomycin							90.2	9.8										0
Doxycycline								87.8	2.4					9.8				9.8
Sulphamethox										90.2	2.4						7.3	7.3
Trimethoprim						92.7	2.4							4.9				4.9
Ciprofloxacin			92.7	7.3														0
Nalidixic acid								19.5	75.6	2.4	2.4							0
Chloramphenicol									2.4	78.0	19.5							0
Florfenicol									12.2	85.4	2.4							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.

Figure 18. Trends in resistance percentages of *E. coli* O157 isolated in The Netherlands from 1998 - 2004



Resistance data from 1998 to 2004 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. 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 and broilers, respectively. Because of the sampling strategy, this method is inherently insensitive for detecting resistance. If resistance is detected, even at low percentages, it indicates that the number of animals or groups of animals that carry these resistant bacteria is substantial

Escherichia coli

Highlights

The resistance levels of *E. coli* show a tendency to increase in both pigs and broilers. Because commensal *E. coli* is present in all animals and the sample is taken randomly, the tendency of increase in resistance reflects the increased usage of antibiotics in these animals. The increased resistance is predominantly observed for the older antibiotics classes (amoxicillin, tetracycline, trimethoprim and sulphonamides). In broilers multiple resistance was substantially more commonly present than in pigs, which may reflect a higher selection pressure, but may also be due to the production system. Broilers only live approximately six weeks, therefore there is limited opportunity for reduction of resistance once selection took place.

In broilers resistance to cefotaxime, indicative of extended spectrum beta-lactamases, was present at a high level. This is intriguing because third-generation cephalosporins are not used in poultry, therefore other selective determinants must exist. ESBLs are often located on integrons linked to other resistance genes.

Resistance to nalidixic acid was highest in strains from poultry. The selection pressure as a result of treatment with quinolones is reflected in the higher resistance percentages in these animals.

Both in slaughter pigs and broilers, the older classes of antibiotics, amoxicillin, doxycycline, trimethoprim, sulphamethoxazole and chloramphenicol showed the highest resistance levels (table 21). Moreover, the resistance levels in broilers were always higher than those in pigs. In broilers resistance to nalidixic acid was very high (46.3%). One nalidixic acid resistant strain was highly resistant to ciprofloxacin, al other nalidixic acid resistant strains showed reduced susceptibility to ciprofloxacin. Although the resistance levels for nalidixic acid from 1998 to 2004 show a certain annual variation, they show a tendency to increase (Fig. 20).

In broilers resistance to cefotaxime, indicative of extended spectrum beta-lactamases, was strikingly high (9.7%). Because the sample of 300 *E. coli* strains is randomly isolated from caecal samples at slaughter, it strongly indicates that the prevalence of ESBLs in broilers is substantial. This is intriguing because third-generation cephalosporins are not used in poultry, therefore other selective determinants must exist. ESBLs are often located on integrons 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. In *Enterobacteriaceae* ESBLs are plasmid mediated. The *E. coli* strains may therefore be a source for transmission of ESBLs to animal-, or zoonotic human pathogens. The genetic nature of these ESBLs needs to be elucidated.

The resistance levels show a tendency to increase in both animal species in 2004 (Fig. 20). Figure 19 shows that in broilers (73%) multiple resistance to three or more antibiotics was substantially higher than in pigs (41%). This may reflect difference in use patterns of antibiotics in these animals but may also be caused by the husbandry systems. Broiler fattening takes approximately six weeks, while pig fattening takes about six months. Therefore in pigs after selection of resistance before and during weaning, a reduction of resistance can occur during the months of fattening. In Sweden multiple resistance is much less common, the levels are 15% in pigs and 5% in chickens (SVARM 2003 and 2004).

Table 21. MIC distributions (in %) for *E. coli* isolated from slaughter pigs (N = 296) and broilers (N = 300) in The Netherlands in 2004.

Pigs (296)	MIC % distributions (µg/ml)																	R%
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	
Amoxicillin							2.0	14.5	37.5	19.9	0.7			25.3				25.3
Cefotaxime				98.6	0.7	0.3		0.3										0.3
Imipenem				91.2	8.8													0
Gentamicin					10.1	57.1	26.7	5.1	0.3	0.3			0.3					0.3
Neomycin							64.5	26.0	7.4				1.0	0.7	0.3			2.0
Doxycycline						0.7	7.8	24.7	2.0	1.0	1.7		12.2	49.7				63.7
Sulphamethox										47.0	0.3						52.7	52.7
Trimethoprim						47.3	9.1	0.3	0.3	0.3				42.6				42.6
Ciprofloxacin			96.6	2.0	0.3	0.7	0.3											0
Nalidixic acid								42.2	54.7	1.4				0.7	1.0			1.7
Chloramphenicol									3.4	77.7	6.8	2.7	5.1	2.4	2.0			12.2
Florfenicol								0.3	20.6	69.6	8.1	1.4						1.4

Broilers (300)	MIC % distributions (µg/ml)																	R%
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	
Amoxicillin								5.0	18.7	12.0	0.3			63.7				63.9
Cefotaxime				87.7	2.7			2.0	1.0	1.3	1.0	4.3						9.7
Imipenem				88.0	11.7													0
Gentamicin					2.7	62.7	24.7	4.7	0.3	0.7	2.3	1.3	0.7					4.3
Neomycin							62.7	20.0	3.7	0.3	0.7	5.0	4.3	2.3	1.0			12.7
Doxycycline						0.3	5.7	21.7	5.7			0.3	19.7	46.7				66.7
Sulphamethox										27.3						0.3	72.3	72.7
Trimethoprim						30.0	7.3							62.7				62.7
Ciprofloxacin			54.7	4.3	26.3	10.7	3.3			0.3								0.3
Nalidixic acid								26.3	26.0	1.3		1.0	1.3	14.7	29.3			46.3
Chloramphenicol									3.0	54.0	20.0	1.7	1.3	0.3	19.7			23.0
Florfenicol									12.7	75.0	10.3	1.0	0.7	0.3				2.0

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

Figure 19. Percentages of *E. coli* strains fully susceptible, resistant to one, two, three, four and more than four antibiotics in pigs and poultry in The Netherlands in 2004.

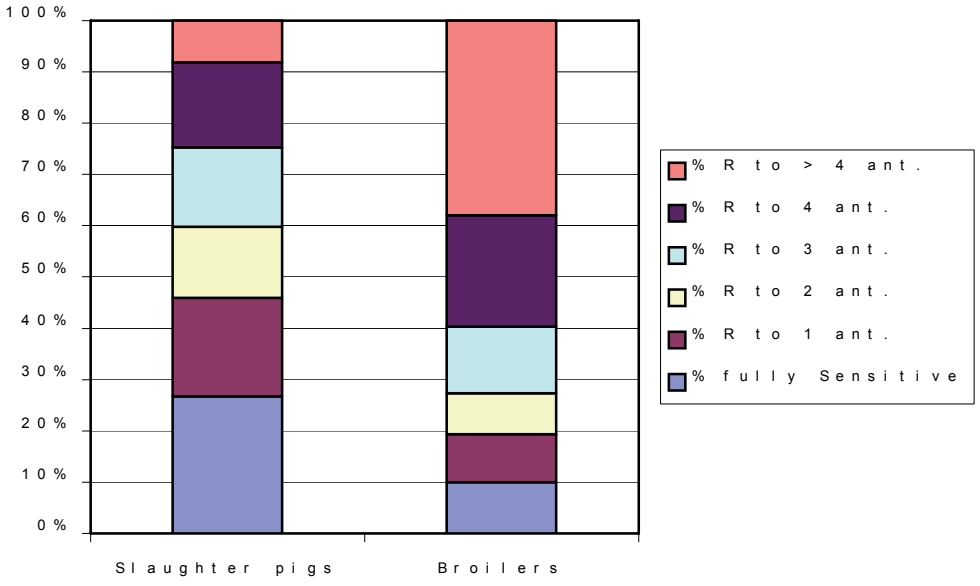
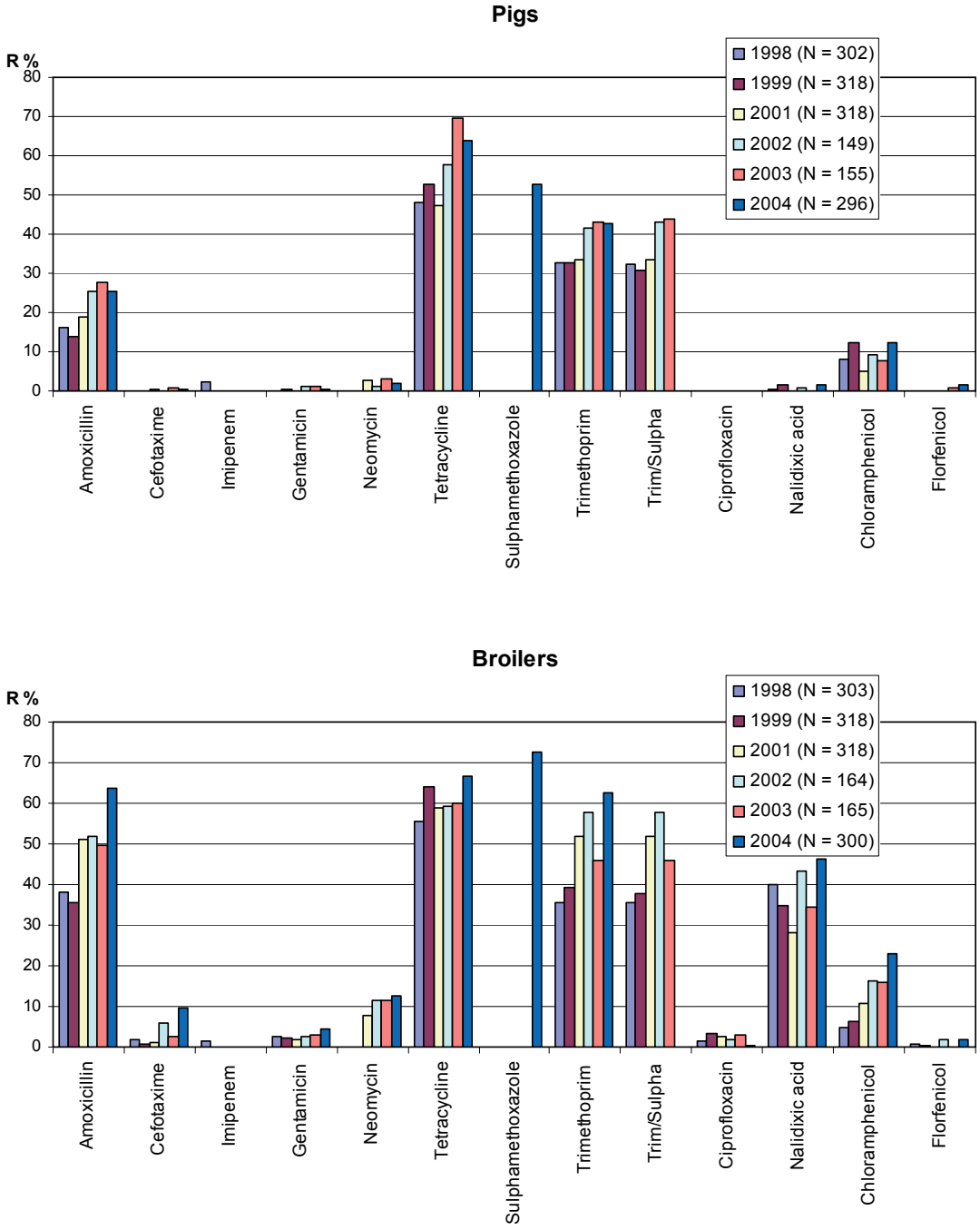


Figure 20. Trends in resistance percentages of *E. coli* isolated from slaughter pigs and broilers in The Netherlands from 1998 - 2004



E. coli in raw meat products of food-animals

Table 22. Resistance % of *E. coli* isolated from raw meat products of poultry, beef and pork in The Netherlands in 2004

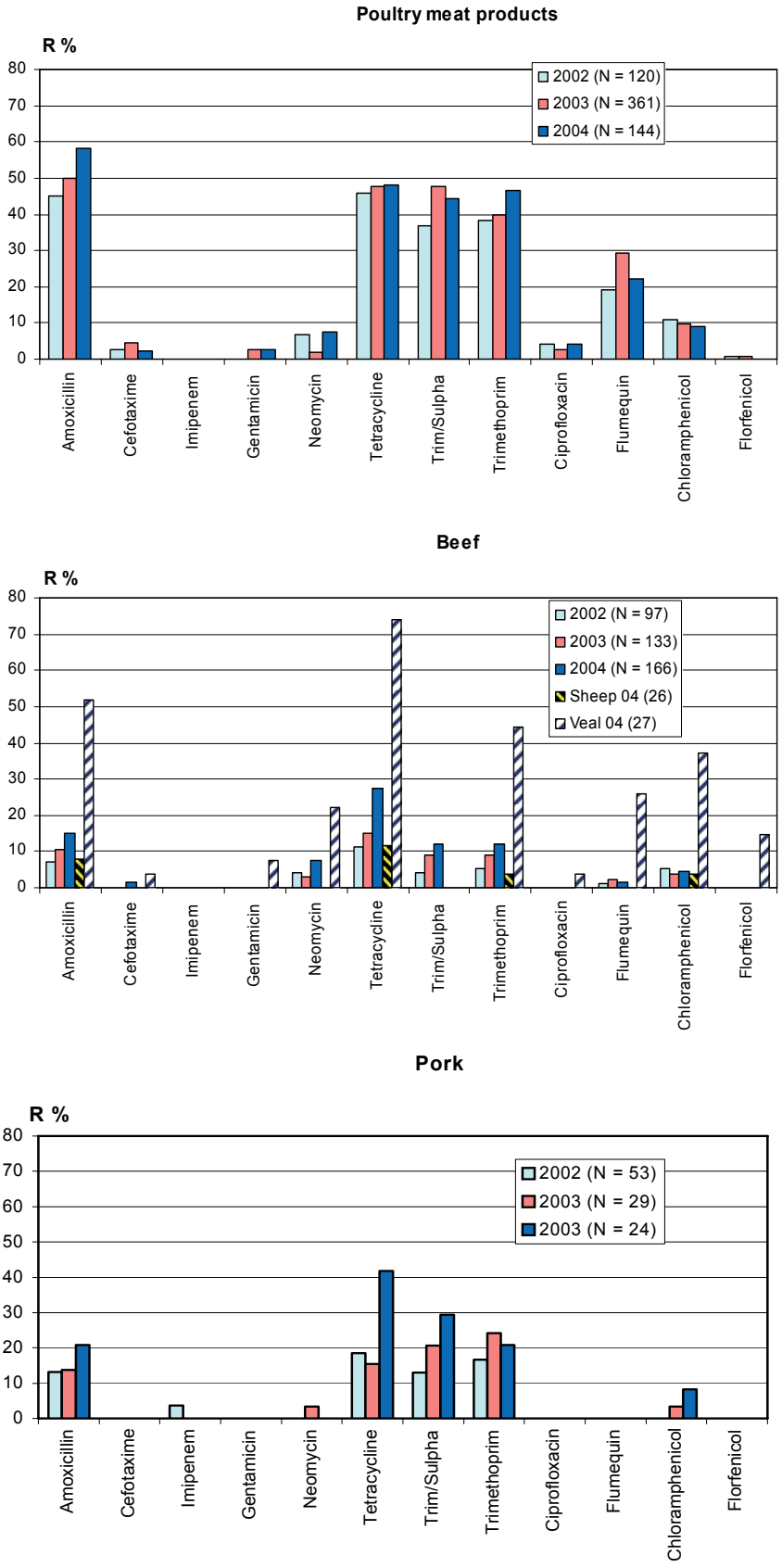
	Poultry	Bio-Chicken	Beef	Veal	Pork	Sheep
	N = 144	N = 41	N = 166	N = 27	N = 24	N = 26
Amoxicillin	58.3	22	15.2	51.9	20.8	7.7
Cefotaxime	2.1	0	1.5	3.7	0	0
Imipenem	0	0	0	0	0	0
Gentamicin	2.8	0	0	7.4	0	0
Neomycin	7.6	0	7.6	22.2	0	0
Tetracycline	47,9	31.7	27.3	74.1	41.7	11.5
Trim/Sulpha	44.4	7.4	12.1	-	29.4	-
Trimethoprim	46.5	12.2	12.1	44.4	20.8	3.8
Ciprofloxacin	4.2	0	0	3.7	0	0
Flumequine	22.2	4.9	1.5	25.9	0	0
Chloramphenicol	9	2.4	4.5	37	8.3	3.8
Florfenicol	0	0	0,0	14.8	0,0	0

Resistance percentages of *E. coli* strains isolated from poultry products and pork (table 22) are very similar to those of isolates from broilers and pigs at slaughter (table 21, fig. 20), indicating that faecal contamination of poultry carcasses is an important factor in the transmission of *E. coli*. In *E. coli* from beef and sheep products, resistance percentages are lower to those in *E. coli* from pork; resistance in veal is at a substantially higher level.

Resistance to flumequine was highest in strains from poultry products and veal, low in beef and not present in pork and sheep. Fluoroquinolones have been licensed for group treatment in broilers and veal calves since 1987 and flumequine since 1981. The selection pressure as a result of this type of treatment is reflected in the higher resistance percentages in these animals.

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.

Figure 21. Trends in resistance percentages of *E. coli* isolated from raw meat products of poultry, cattle and pigs in The Netherlands from 2002 – 2004 and of sheep and veal calves in 2004.



Enterococcus faecium, Enterococcus faecalis

In 2004 *E. faecalis* was included in the monitoring programme. From each sample taken at slaughterhouses inoculated on Slanetz and Bartley agar, after incubation at 42°C, both a colony typical for *E. faecium* and one typical for *E. faecalis* was selected. Further determination was done by PCR. The reason for the inclusion of *E. faecalis* was that after 1999, when most of the growth promoters were banned, a slow but constant decrease in isolation rate for *E. faecium* from faecal samples was observed. Because differences in intrinsic susceptibility exist between the two species for a.o. flavomycin and the streptogramins, the data will be reported separately.

Highlights

In 2004 *E. faecalis* was included in the monitoring programme. The reason was that the isolation rates of *E. faecium* decreased after 1999, the year of the partial ban of growth promoters. In slaughter pigs the resistance levels in *E. faecium* remained stable in 2004 as compared to 2003 (Fig. 13). In broilers resistance to doxycycline, erythromycin, streptomycin and salinomycin showed a slow tendency to increase.

Multiple resistance to three or more antibiotics was commonly present and much more common in strains from broilers than in strains from pigs.

Resistance percentages in *E. faecium* isolated from raw meat products were lower than those found in isolates from food-animals. This may be selection bias due to the relatively small numbers tested. It may also indicate that subpopulations of strains adapted to survival in meat products exist.

Vancomycin resistance was only found in *E. faecalis* isolated from beef. Resistance levels in *E. faecalis* from veal were similar as those in poultry products. Resistance in biologically reared poultry, and sheep was lower than in other food animals.

E. faecalis is intrinsically reduced susceptible to quinu/dalfopristin (table 23). The breakpoint R > 2 µg/ml is not adequate for this species and should be > 32 µg/ml to distinguish the native from the resistant population. *E. faecium* is intrinsically high level resistant to flavomycin. The fact that the resistance levels for flavomycin presented in this chapter are not always 100% may be due to inadequate identification or genetic variations within the *E. faecium* population.
Avilamycin was not included in the 2004 test panel because the producer refused to provide this active substance. The reason was that the broth microdilution method used is not validated for avilamycin.

In *E. faecalis* and *E. faecium* strains isolated from broilers and pigs, next to doxycycline, the highest resistance percentages were found for those antibiotics representing the growth promoters: bacitracin, erythromycin representing tylosin and spriramycin, and quinu/dalfopristin (Synercid®) (streptogramins) and salinomycin (ionophore) (tables 23 and 24). Resistance to the glycopeptide vancomycin was only detected in *E. faecium* from broilers (R = 1.1 %). Amoxicillin resistance was only detected in *E. faecium* and ciprofloxacin resistant strains were not detected. High-level streptomycin resistant strains were present in both animal and bacterial species.

In pigs the resistance levels seemed to be stable, whereas in broilers the resistance levels show a tendency to increase (Fig. 22) . Multi drug resistance is commonly present in strains from both animal species, but substantially more common in broilers than in pigs. In broilers 70% of *E. faecalis* and >90% of *E. faecium* was resistant to three or more antibiotic classes, while in pigs this was 55% and 62%, respectively (Fig. 23).

Table 23. MIC distributions (In %) for *E. faecalis* isolated from slaughter pigs (N = 35) and broilers (N = 110) in The Netherlands in 2004.

Pigs N = 35	**MIC % distribution (µg/ml)**															**R (%)**
	0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	
Amoxicillin				100												0
Bacitracin								2.9	5.7	34.3	34.3	5.7	17.1			22.9
Chloramphenicol							74.3	17.1		8.6						8.6
Ciprofloxacin			8.6	88.6	2.9											0
Doxycycline		11.4	8.6			2.9	31.4	31.4	14.3							77.1
Erythromycin				28.6	22.9					2.9		45.7				48.6
Flavomycin						100										0
Genta > 500											97.1			2.9		2.9
Linezolid			2.9		94.3	2.9										0
Salinomycin			8.6	65.7	11.4	2.9	11.4									11.4
Strep > 2000													62.9	2.9	34.3	34.3
Quinu/dalfopristn						2.9	17.1	77.1	2.9							100*
Vancomycin				77.1	22.9											0

Broilers N = 110	**MIC % distribution (µg/ml)**															**R (%)**
	0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	
Amoxicillin				99.1	0.9											0
Bacitracin								0.9	10.0	7.3	41.8	7.3	32.7			40.0
Chloramphenicol						5.5	80.9	9.1		4.5						4.5
Ciprofloxacin			18.2	78.2	3.6											0
Doxycycline		7.3	13.6	0.9		2.7	40.0	27.3	8.2							75.5
Erythromycin				31.8	9.1	1.8	2.7	2.7	5.5	2.7		43.6				57.3
Flavomycin						97.3	2.7									0
Genta > 500											100					0
Linezolid			0.9	3.6	95.5											0
Salinomycin			4.5	42.7	7.3	10.9	34.5									34.5
Strep > 2000													72.7	2.7	24.5	24.5
Quinu/dalfopristin			1.8			0.9	25.5	67.3	3.6	0.9						98.2*
Vancomycin				59.1	40.9											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.
* *E. faecalis* is intrinsically decreased susceptible to streptogramins, therefore the R% data for quinu/dalfopristin (Synercid®) represent an overestimation of the resistant population.

Table 24. MIC distributions (In %) for *E. faecium* isolated from slaughter pigs (N = 121) and broilers (N = 180) in The Netherlands in 2004.

Pigs N =121	MIC % distribution (µg/ml)															R (%)
	0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	
Amoxicillin				48.8	47.1	3.3	0.8									0
Bacitracin						19.0	7.4		1.7	2.5	22.3	21.5	25.6			47.1
Chloramphenicol							91.7	7.4	0.8							0
Ciprofloxacin			20.7	49.6	13.2	14.9	1.7									0
Doxycycline		22.3	1.7		0.8	0.8	7.4	38.8	27.3	0.8						74.4
Erythromycin				15.7	42.1	9.9	5.8	1.7				24.8				32.2
Flavomycin													0.8	99.2		100*
Genta > 500											100.0					0
Linezolid					79.3	19.8	0.8									0.8
Salinomycin				21.5	33.1	1.7	31.4	12.4								43.8
Strep > 2000													83.5	8.3	8.3	8.3
Quinu/dalfopristin			5.8	5.8	35.5	44.6	8.3									52.9
Vancomycin			81.8	14.0	2.5	1.7										0

Broilers N = 180	MIC % distribution (µg/ml)															R (%)
	0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	
Amoxicillin				42.2	37.2	15.6	0.6	0.6	2.2	1.7						4.5
Bacitracin					0.6	1.1	3.9	6.7	1.7	0.6	7.8	20.6	57.2			77.8
Chloramphenicol						2.8	74.4	13.9	8.9							0
Ciprofloxacin			1.7	10.6	25.6	54.4	7.8									0
Doxycycline		21.7	0.6	0.6	2.8	1.1	23.3	17.2	32.8							73.3
Erythromycin				18.3	8.3	3.9	1.1		0.6	0.6	0.6	66.7				69.4
Flavomycin												0.6	7.2	92.2		100*
Genta > 500											96.1	1.1	0.6	2.2		2.2
Linezolid				1.7	87.8	10.6										0
Salinomycin				6.1	8.3	3.9	78.9	2.8								81.7
Strep > 2000													66.1	1.7	32.2	32.2
Quinu/dalfopristin			6.7	12.8	10.6	48.3	8.9	8.3	4.4							70.0
Vancomycin			73.3	16.7	8.3	0.6				0.6	0.6					1.1

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the breakpoint.
* Intrinsic resistance to flavomycin.

Figure 22. Trends in resistance percentages of *E. faecium* isolated from slaughter pigs and broilers in The Netherlands from 1998 - 2004

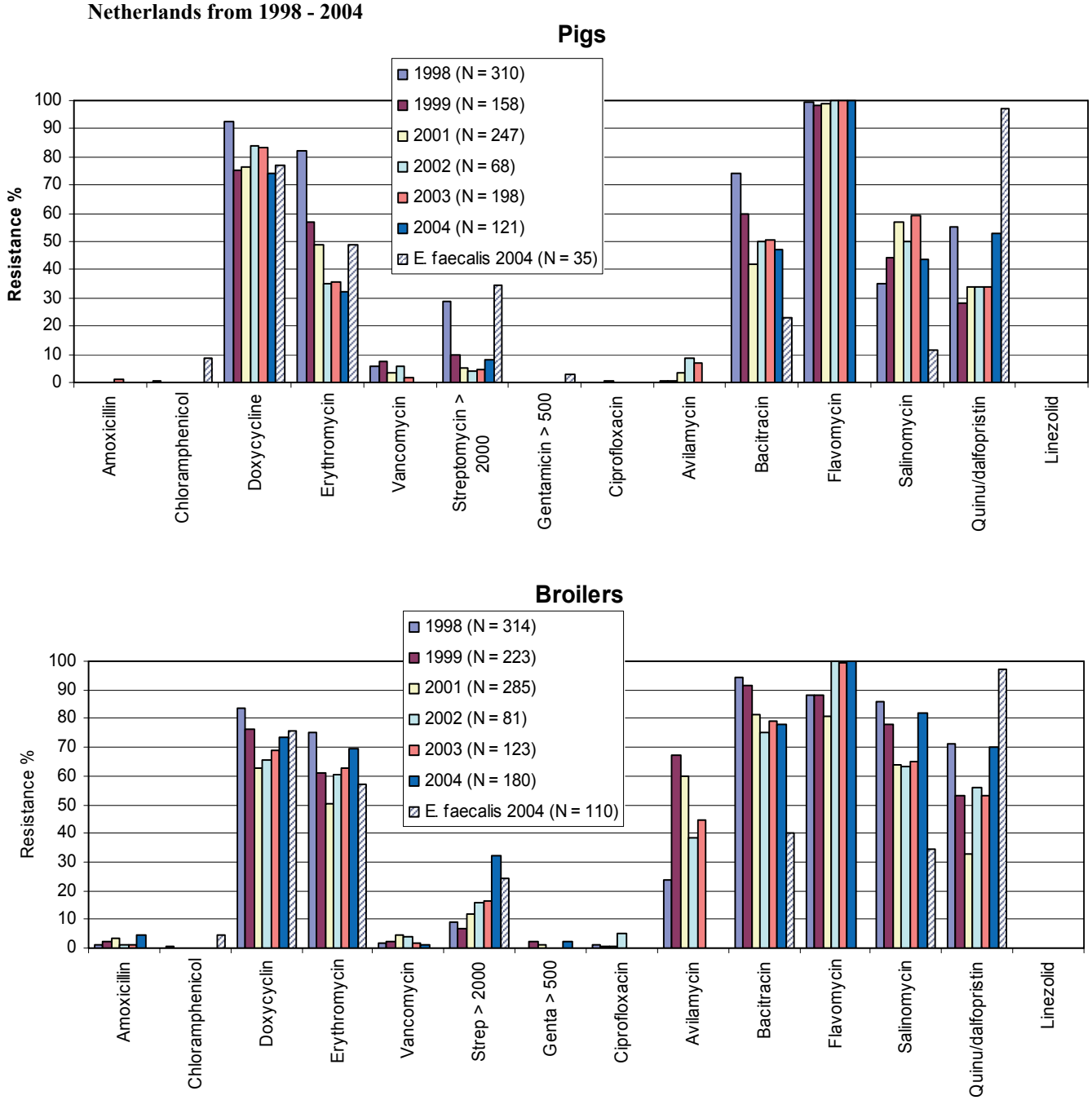
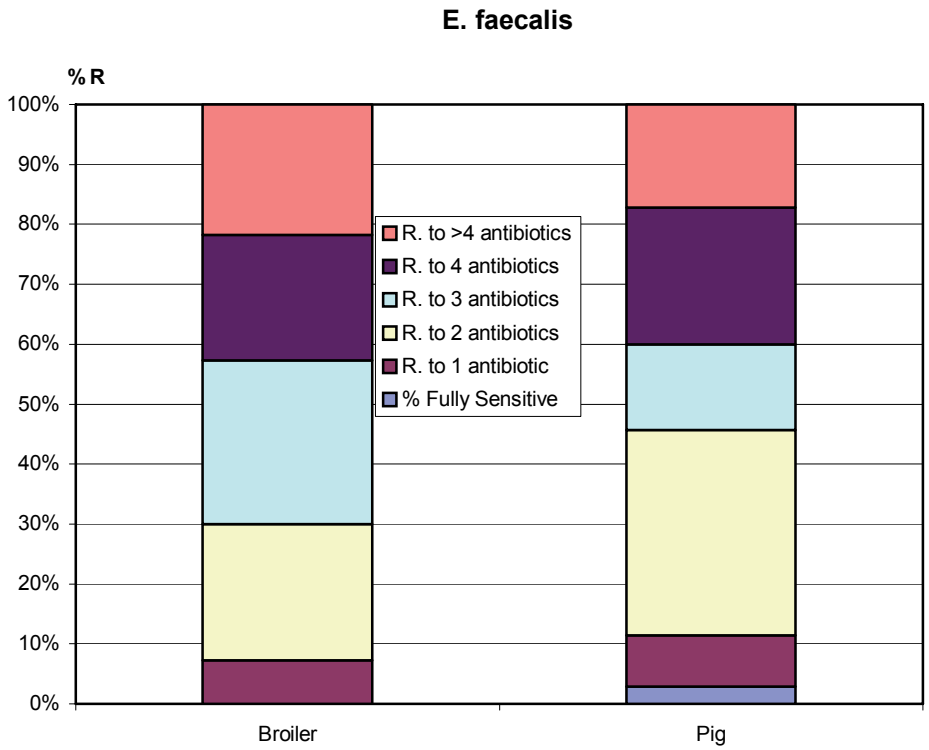
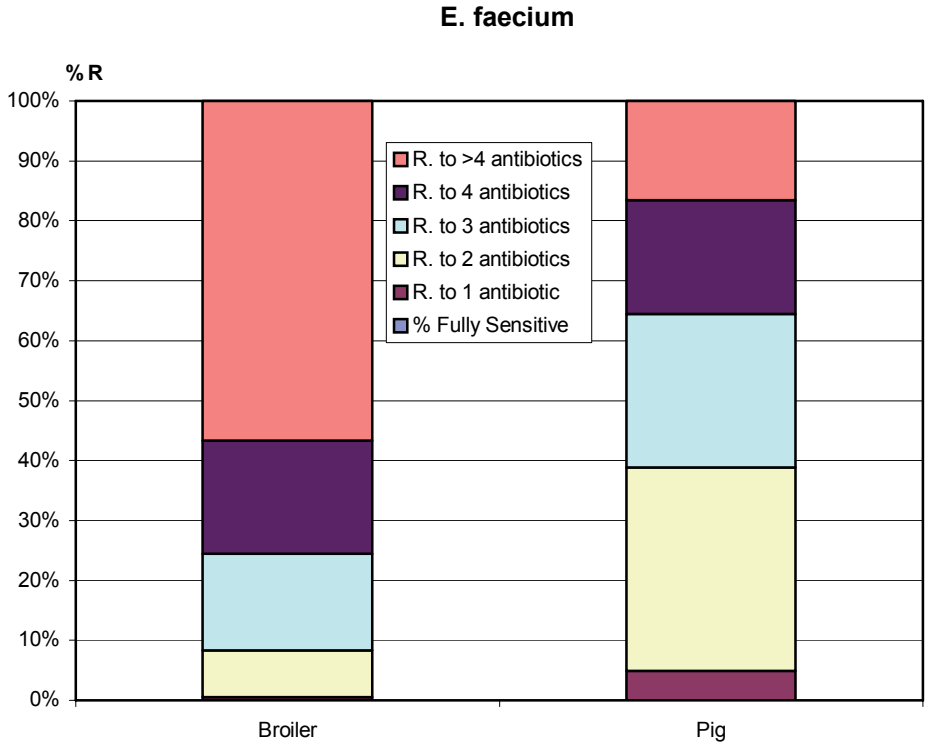


Figure 23. Percentages of *E. faecalis* and *E. faecium* strains fully susceptible, resistant to one, two, three, four and more than four antibiotics in pigs and poultry in The Netherlands in 2004.





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, bio-chickens, beef, veal, sheep and pork in the Netherlands in 2004

E. faecalis	**Poultry N = 24**	**Bio-chicken N = 17**	**Beef N = 130**	**Veal N = 37**	**Sheep N = 25**	**Pork N = 61**
Amoxicillin	0	0	0	0	0	0
Bacitracin	33.3	35.3	9.9	35.1	40	4.9
Ciprofloxacin	0	0	1.4	0	0	3.3
Doxycycline	62.5	29.4	22.2	45.9	28	20
Erythromycin	41.7	14.3	12.5	32.4	8	1.6
Flavomycin	8.3	0	5.6	8.1	4	1.6
Gentamicin > 500 µg/ml	4.2	0	2.8	5.4	0	0
Linezolid	-	-	-	0	0	-
Salinomycin	0	-	0	0	0	0
Streptomycin > 1000 µg/ml	12.5	-	10.8	-	-	0
Streptomycin > 2000 µg/ml	8.3	0	7	17.1	0	0
Quinu/dalfopristin	100*	100*	86.1*	89.2*	56.0*	82.0*
Vancomycin	0	0	1.4	0	0	0
E. faecium	**Poultry N = 42**	**Bio-chicken N = 20**	**Beef N = 46**	**Veal N = 12**	**Sheep N = 20**	**Pork N = 17**
Amoxicillin	4.8	0	0	0	0	0
Bacitracin	61	15	17.4	0	5	5.9
Ciprofloxacin	0	10	0	0	-	0
Doxycycline	57.1	20	17.4	8.3	10	29.4
Erythromycin	57.1	5	10.9	25	5	17.6
Flavomycin	73.2*	60*	100*	100*	85*	82.4*
Gentamicin > 500 µg/ml	0	15	0	0	0	0
Linezolid	-	-	-	0	0	-
Salinomycin	9.8	0	4.3	0	0	11.8
Streptomycin > 1000 µg/ml	24.4	0	4.3	-	-	0
Streptomycin > 2000 µg/ml	9.8	-	2.2	0	5.6	0
Quinu/dalfopristin	19.0	11.1	0	0	5	5.9
Vancomycin	0	0	0	0	0	0

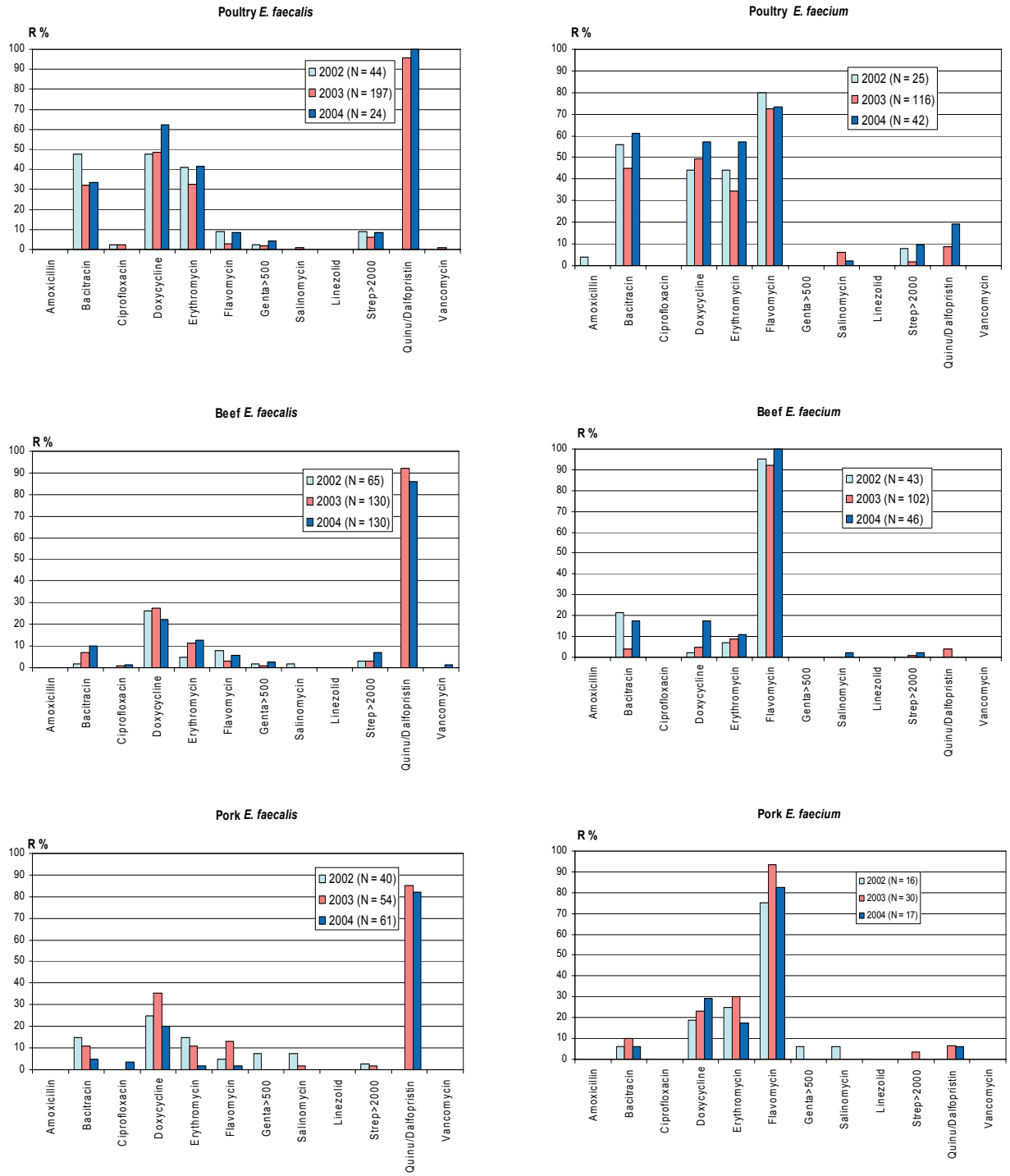
* *E. faecalis* is intrinsically decreased susceptible to streptogramins, therefore the R% data for quinu/dalfopristin (Synercid®) represent an overestimation of the resistant population. *E. faecium* is intrinsically resistant to flavomycin.

Resistance percentages in *E. faecium* isolated from raw meat products are lower than those found in isolates from food-animals. This may be selection bias due to the relatively small numbers tested. It may also indicate that subpopulations of strains adapted to survival in meat products exist.

Vancomycin resistance was only found in *E. faecalis* isolated from beef. Resistance percentages in isolates from beef were lower than those from the poultry and pig products. Resistance levels in *E. faecalis* from veal were similar as those in poultry products.
Resistance levels in *E. faecalis* were similar to those from *E. faecium* except for bacitracin and doxycycline from cattle.
Resistance in biologically reared poultry was in general lower than in broilers. Surprisingly two *E. faecium* strains from bio-chickens showed high level resistance to ciprofloxacin.

Fig 16 shows the trend from 2002 to 2004. Real trends cannot be observed and trend analysis is complicated by the relatively small numbers of strains per year.

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 2002 to 2004



Listeria monocytogenes

All strains isolated from 2001 to 2004 and sent to the National Institute of Public Health and the Environment (RIVM), Bilthoven for confirmation and typing (N = 146) were tested for susceptibility using broth microdilution. The origin of the strains was predominantly human; 55% were isolated from blood samples and 25% from liquor. The remaining 20% was isolated from environmental specimens and various food products.
The purpose of this study was to determine the susceptibility level of *Listeria* spp. to a wide variety of antimicrobial agents used in human and veterinary medicine.
The strains were tested for susceptibility to amoxicillin, neomycin, gentamicin, tetracycline, doxycycline, erythromycin, ciprofloxacin, chloramphenicol, florfenicol, imipenem, sulphamethoxazole, trimethoprim, linezolid, salinomycin, quinu/dalfopristin and vancomycin.

Highlights
The strains were all susceptible to all antibiotics listed, except 6 that were resistant to sulphamethoxazole (MIC > 1024 µg/ml).

Animal pathogens

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 the antibiotics included in the panel. The coliform bacteria (*Enterobacter, Klebsiella* and other species) showed a high level of resistance to amoxicillin, and to the combination with clavulanic acid. The *S. aureus* isolates tested were susceptible to most antibiotics, 12.1% were penicillin resistant. Oxacillin resistance (MRSA) was not present. The coagulase negative staphylococci were more resistant than *S. aureus,* 40.8% were resistant to penicillin and 6.1% to oxacillin (*mecA*-positive). In the streptococci only resistance to erythromycin, lincomycin, pirlimycin and tetracycline was observed. In 2004 *S. uberis* was more frequently resistant to erythromycin, lincomycin and pirlimycin than *S. dysgalactiae*. Resistance to tetracycline was highest in *S. dysgalactiae*.

Table 26. 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 2004.

***E. coli* (N = 101)**	**MIC % distribution (µg/ml)**															**R%**
	0.015	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	
Amoxicillin							2.0	19.8	52.5	10.9	1.0		13.9			13.9
Amox-clavulanic acid							3.0	32.7	45.5	15.8	3.0					0
Cefquinome			92.1	7.9												0
Cefoperazone			5.9	35.6	33.7	9.9	4.0	6.9	3.0	1.0						0
Cefuroxime								13.9	50.5	34.7	1.0					0
Tetracycline							35.6	45.5	4.0				14.9			14.9
Gentamicin					5.0	57.4	30.7	6.9								0
Kanamycin								16.8	58.4	18.8		5.9				5.9
Neomycin						3.0	68.3	18.8	4.0		1.0	5.0				5.0
Streptomycin									14.9	63.4	5.9		5.9	9.9		15.8
Enrofloxacin		70.3	28.7							1.0						1.0
Trim/Sulphamethoxazole				85.1	4.0	2.0						8.9				8.9

Coliform (N = 88)	**MIC % distribution (µg/ml)**															**R%**
	0.015	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	
Amoxicillin								9.1	6.8	1.1	1.1	1.1	80.7			81.8
Amox-clavulanic acid							6.8	54.5	9.1	2.3	1.1	1.1	22.7	2.3		26.1
Cefquinome			75.0	17.0	6.8	1.1										0
Cefoperazone			1.1	10.2	31.8	19.3	10.2	14.8	9.1	3.4						0
Cefuroxime							4.5	34.1	28.4	10.2	6.8	15.9				15.9
Tetracycline						3.4	26.1	45.5	10.2	1.1	1.1	3.4	9.1			13.6
Gentamicin				1.1	38.6	52.3	8.0									0
Kanamycin							6.8	53.4	25.0	4.5	1.1	9.1				9.1
Neomycin					2.3	28.4	59.1	5.7	3.4	1.1						0
Streptomycin								5.7	63.6	19.3	1.1	2.3	3.4	4.5		7.9
Enrofloxacin		31.8	52.3	10.2	4.5	1.1										0
Trim/Sulphamethoxazole				64.8	28.4	3.4	2.3					1.1				1.1

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. 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 amoxicillin, streptomycin, trim/sulpha and tetracycline was present in significant percentages. All strains were susceptible to the 2nd (cefuroxime) and 3rd generation cefalosporins (cefquinome and cefoperazone) tested, although the cefoperazone MICs show a wide variation in the level of the susceptibilities. One isolate was resistant to enrofloxacin. In comparison with the commensal *E. coli'*s from food animals often showing subpopulations with decreased susceptibility to fluoroquinolones, the one resistant isolate was high-level resistant to fluoroquinolones. All isolates were susceptible to gentamicin.

The coliform bacteria (29 *Enterobacter ,* 48 *Klebsiella,* 11 other species) showed a high level of resistance to amoxicillin (almost all klebsiella's are ß-lactamase producers), and to the combination with clavulanic acid (predominantly *Enterobacter* and other species). The coliform bacteria produced beta-lactamases that were in 15.9% of the cases resistant to the second-generation cephalosporin cefuroxime but were always susceptible to the third-generation cephalosporins. Fig. 25 shows that from 2002 to 2004 the resistance levels are stable.

Figure 25. Trends in resistance percentages for *E. coli* and coliform bacteria isolated from clinical mastitis cases in dairy cattle in the Netherlands from 2002 – 2004.

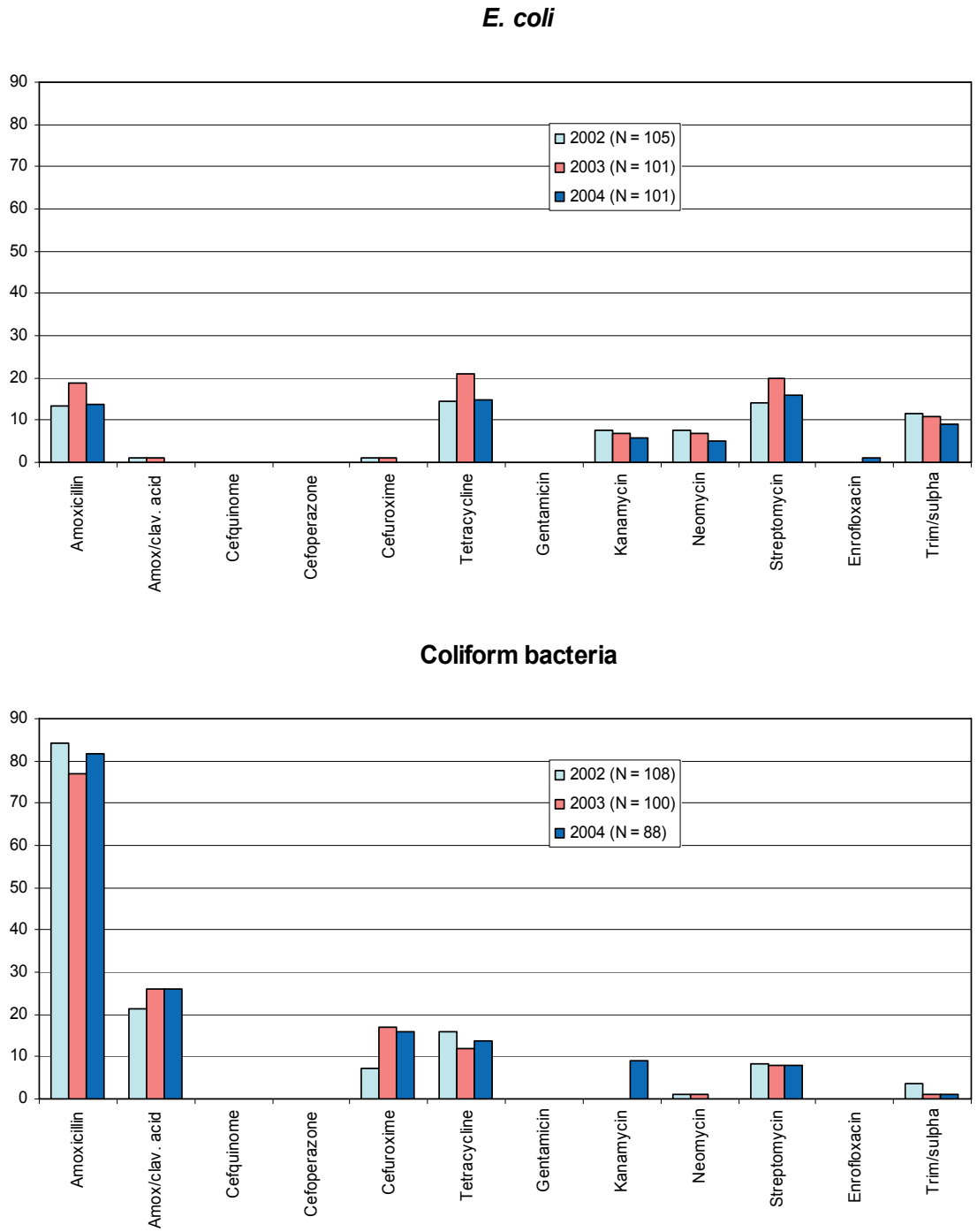


Table 27. MIC-distributions (in %) of *S. aureus* and coagulase-negative staphylococci isolated from clinical mastitis cases in dairy cattle by the Animal Health Service in Deventer in 2004.

***S. areus* (N = 99)**	**MIC % distributions (µg/ml)**																**R%**
	0.015	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	
Penicillin			85.9	2.0			2.0	1.0	4.0	3.0	2.0						12.1
Oxacillin				26.3	35.4	35.4	3.0										0
Amox-clavulanic acid				58.6	28.3	4.0	9.1										0
Cephalothin				26.3	45.5	24.2	3.0	1.0									0
Tetracycline					7.1	89.9	1.0			1.0	1.0						1.0
Kanamycin						1.0	3.0	48.5	43.4	4.0							0
Neomycin				1.0	13.1	62.6	22.2	1.0									0
Streptomycin									24.2	60.6	13.1			2.0			2.0
Erythromycin					68.7	30.3	1.0										0
Lincomycin						3.0	90.9	1.0			1.0	2.0	2.0				5.1
Pirlimycin					18.2	63.6	15.2		3.0								3.0
Trim/sulpha				96.0	3.0	1.0											0
Coagulase neg. Staphylococci (N = 98)	**MIC % distributions (µg/ml)**																**R%**
	0.015	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	
Penicillin			52.0	7.1	11.2		3.1	9.2	6.1	4.1	7.1						40.8
Oxacillin			2.0	19.4	42.9	23.5	6.1	3.1	1.0	1.0	1.0						6.1*
Amox-clavulanic acid				44.9	33.7	15.3	5.1	1.0									0
Cephalothin			2.0	18.4	51.0	21.4	6.1	1.0									0
Tetracycline				3.1	36.7	35.7	2.0	2.0	3.1	1.0	2.0	14.3					16.3
Kanamycin				1.0	18.4	30.6	29.6	13.3	6.1			1.0					1.0
Neomycin				77.6	16.3	3.1	2.0		1.0								0
Streptomycin						3.1	13.3	30.6	25.5	12.2	4.1	4.1	4.1	3.1			11.3
Erythromycin				6.1	46.9	40.8	1.0		1.0	1.0	1.0	2.0					4.1
Lincomycin					4.1	29.6	38.8	8.2	5.1	3.1	2.0	2.0	7.1				14.3
Pirlimycin				18.4	51.0	19.4	3.1	3.1	3.1			2.0					5.1
Trim/sulpha				43.3	43.3	13.4											0

The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. The vertical bars indicate the breakpoints.
* all strains with MIC ≥ 2 µg/ml (6.1%) were *mecA*-positive

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 2004 12.1% of the isolates were penicillinase producers but oxacillin resistance was not present, 5.1% were resistant to lincomycin and 3% to the related but more potent lincosamide drug, pirlimycin.

The coagulase negative staphylococci were more resistant than *S. aureus.* In 2004, 40.8% were resistant to penicillin and 35.7% to oxacillin using the breakpoint 0.25 µg/ml. CLSI standard M31-A2 prescribes for oxacilline as breakpoint R ≥ 4 µg/ml. A study done in the EU-project ARBAO-II, coordinated by the Danish Institute for Food and Veterinary Research demonstrated that using the R breakpoint ≥ 4 µg/ml would lead to an underestimation of *mecA* (the gene encoding for oxa/methicillin resistance) positive strains. However, using the breakpoint prescribed in CLSI standard M100-S15 intended for human medicine for coagulase negative staphylococci, R ≥ 0.5 µg/ml lead to an overestimation of *mecA*-positive strains. Therefore it was suggested that all strains with oxacillin MICs ≥ 4 µg/ml and those with MICs ≥ 0.5 µg/ml but PCR confirmed *mecA*-positive should be classified resistant. Using this method the resistance percentage for oxacillin was 6.1%; six strains were *mecA*-positive, their 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 26. Trends in resistance percentages for *S. aureus* and coagulase negative staphylococci isolated from mastitis milk in The Netherlands from 2002 - 2004.

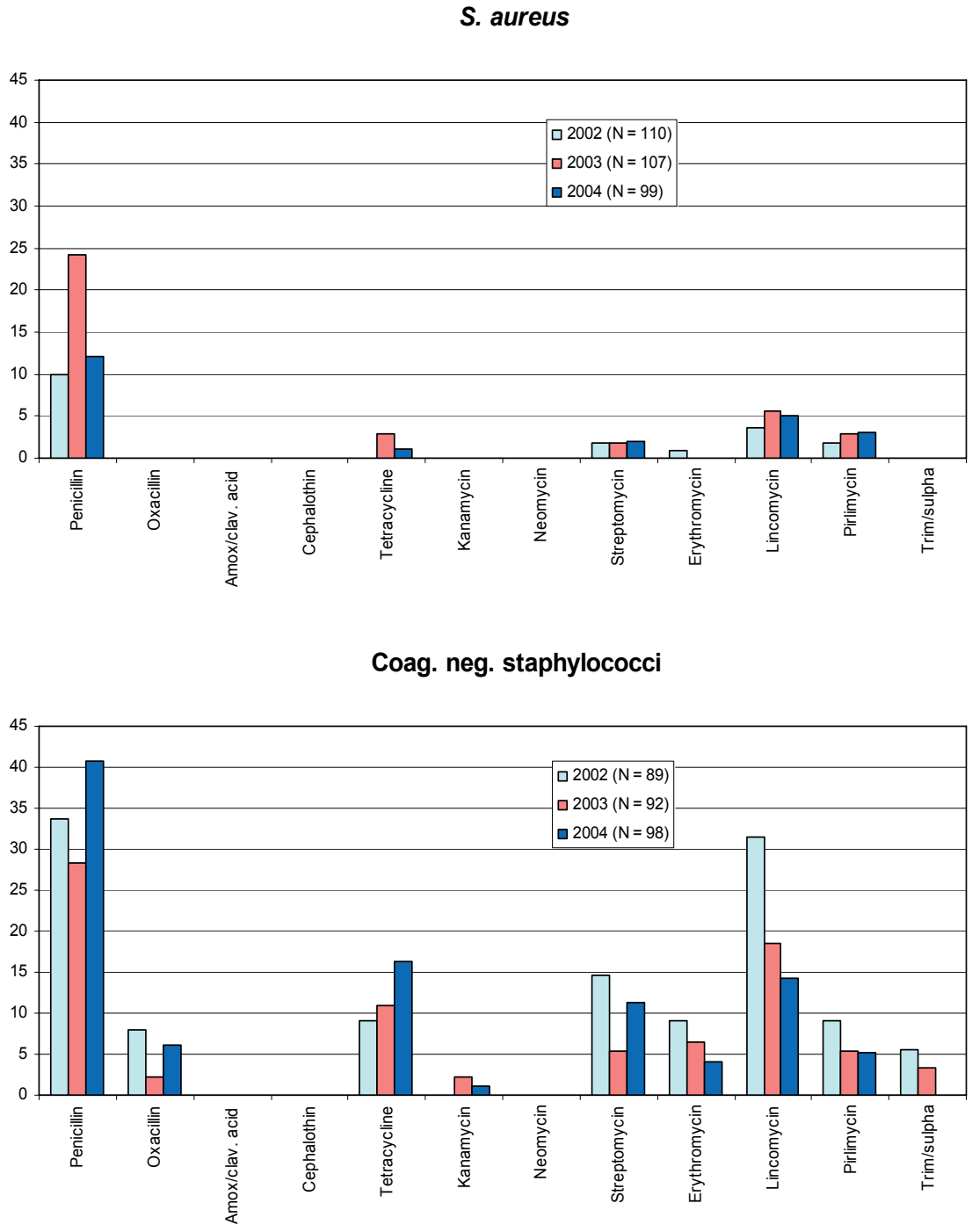


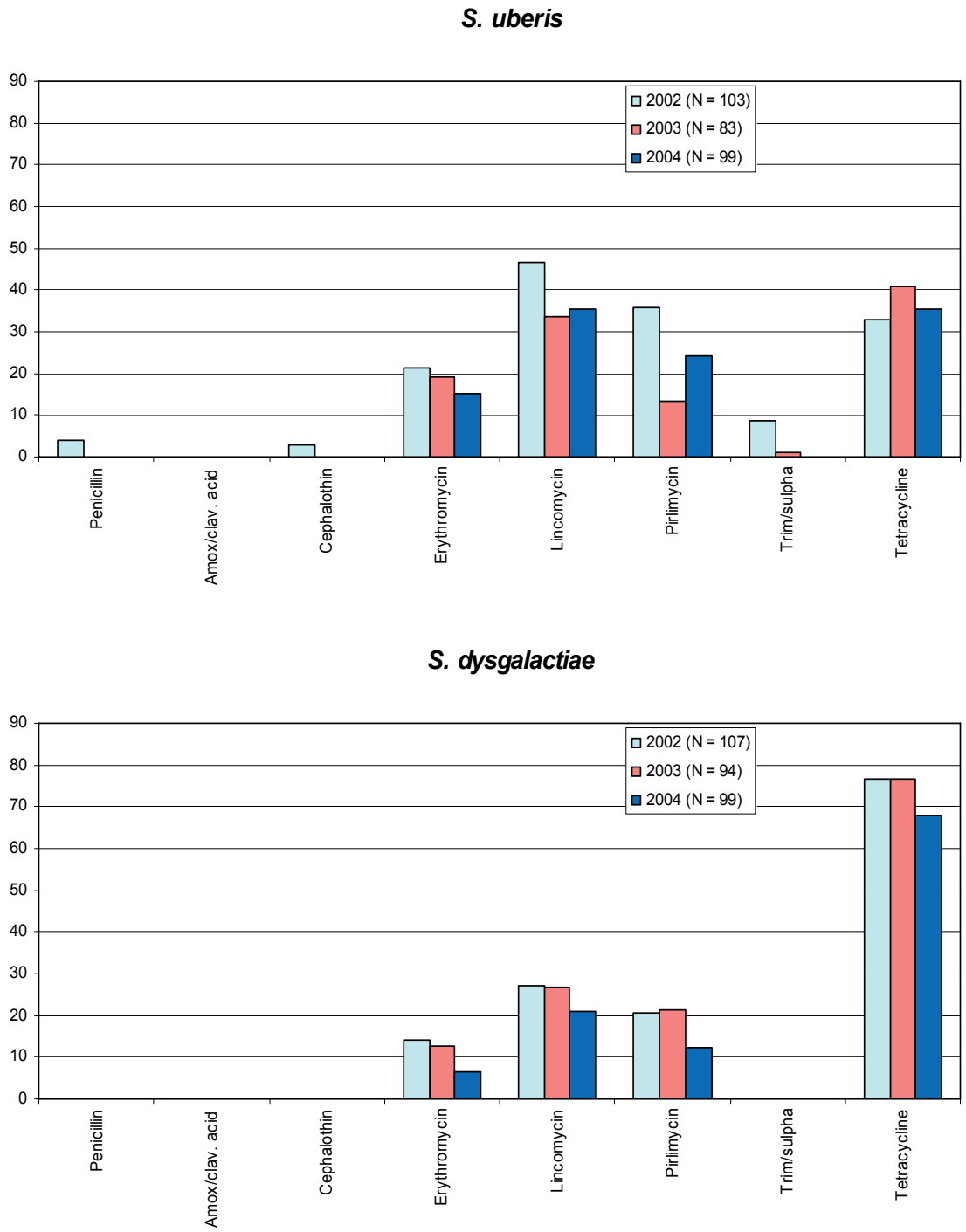
Table 28. MIC-distributions (in %) of S. *uberis* and *S. dysgalactiae* isolated from mastitis milk samples from Dutch cattle by the Animal Health Service in 2004.

	MIC % distribution (µg/ml)															
***S. uberis* (N = 99)**	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Penicillin	49.5	5.1	12.1	20.2	11.1	1.0	1.0									0
Amox/clav. Acid	13.1	35.4	7.1	20.2	23.2			1.0								0
Cephalothin				41.4	14.1	31.3	13.1									0
Erythromycin			53.5	31.3			5.1		2.0				8.1			15.2
Lincomycin				22.2	10.1		1.0	22.2	9.1		1.0	3.0	31.3			35.4
Pirlimycin		2.0	46.5	14.1	1.0	2.0	1.0	9.1	16.2	1.0	2.0	2.0	3.0			24.2
Trim/sulpha			1.0	24.2	58.6	15.2		1.0								0
Tetracycline			0.0	2.0	20.2	36.4	5.1	1.0				20.2	13.1	2.0		35.4
***S. dysgalactiae* (N = 90)**	**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	97.8	1.1	1.1													0
Amox/clav. Acid	94.4	4.4	1.1													0
Cephalothin			20.0	76.7	3.3											0
Erythromycin	1.1	10.0	78.9	3.3									6.7			6.7
Lincomycin				37.8	35.6	1.1		1.1	3.3	7.8		1.1	12.2			21.1
Pirlimycin	1.1	18.9	50.0	16.7		1.1			2.2	2.2	1.1	1.1	5.6			12.2
Trim/sulpha		1.1	7.8	66.7	21.1	3.3										0
Tetracycline						1.1	1.1	11.1	18.9	1.1	1.1	10.0	55.6			67.8

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 2004 *S. uberis* was more frequently resistant to erythromycin, lincomycin and pirlimycin than *S. dysgalactiae*. Resistance to tetracycline was highest in *S. dysgalactiae*.
The observed differences in resistance percentages of the lincosamides and trimethoprim-sulphamethoxazole for *S. uberis* between 2002 and 2004 are striking (fig. 20), but again it may be part of the normal variation and not represent a real trend.

Figure 27. Trends in resistance percentages for *S. uberis* and *S. dysgalactiae* isolated from mastitis milk in The Netherlands from 2002 - 2004.



Enteric pathogens: *Brachyspira hyodysenteriae*

Highlights
Of the strains tested 68.8% was resistant to tylosin and 0% resistant to tiamulin

Table 29. MIC % distribution for *B. hyodysenteriae* isolated from pigs in the Netherlands in 2003 - 2004

N = 16	MIC % distribution (µg/ml)														R%
	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	
Tylosin								25	6.2					68.8	68.8
Tiamulin		68.8	31.3												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.

In 2002 CIDC-Lelystad started with the monitoring of resistance to tylosin and tiamulin in *B. hyodysenteriae* in The Netherlands. The inclusion of this bacterial species in the programme was considered important because of the realistic scenario that this species is becoming resistant to all drugs licensed. Tylosin and tiamulin are included, because they represent all antibiotics used to treat dysentery in pigs. Tylosin is cross resistant with lincomycin and tiamulin with valnemulin. The strains tested are all isolated from animals suffering from swine dysentery at the Animal Health Service in Deventer, The Netherlands. In 2002 all isolates tested were resistant to tylosin, therefore it was surprising to find 5 tylosin (lincomycin) susceptible isolates of *B. hydoysenteriae* in the small collection of strains isolated in 2003/2004. All isolates were susceptible to tiamulin (and therefore also to valnemulin).

Poultry pathogen *Mycoplasma synoviae*

In 2004 a selection of *M. synoviae* strains isolated from specimens taken from diseased poultry in the Netherlands were quantitatively tested for susceptibility to a number of antibiotics available in veterinary medicine. The direct reason was that *M. synoviae* infections in poultry poorly responded to antibacterial therapy and little knowledge existed on the susceptibility of clinical isolates. Mycoplasma's are by nature fastidious organisms and routinely not tested for susceptibility. Moreover validated and well-standardised methodologies are lacking. To test the susceptibility a method adopted from the one described by P.C. Hannan in Veterinary Research in 2000 was used (see appendix. Materials and Methods).

Highlights
All strains were susceptible to doxycycline and the macrolides: tylosin and tilmicosin. Resistant subpopulations existed for the fluoroquinolones. For enrofloxacin the subpopulation with MICs varying from 4 - 16 µg/ml were more clearly separated from the susceptible population than for the related compound difloxacin.

Table 30. MIC % distribution for *M. synoviae* isolated from poultry in the Netherlands in 2003 - 2004

M. synoviae (N = 17)	MIC % distribution												R%
	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	
Doxycycline	-	11.8	35.3	47.1	5.9	-	-	-	-	-	-	-	0
Tylosin	52.9	29.4	5.9	-	5.9	5.9	-	-	-	-	-	-	0
Tilmicosin	-	-	-	35.3	47.1	17.6	-	-	-	-	-	-	0
Enrofloxacin	-	-	-	5.9	23.5	29.4	17.6	-	5.9	11.8	5.9	-	23.5
Difloxacin	-	-	-	-	5.9	29.4	41.2	11.8	11.8	-	-	-	11.8

III Appendices

Appendix I. Materials and Methods

Salmonella spp.

A total of 10.234 isolates were tested for antimicrobial resistance between 1999-2004 (table 31). Human isolates (N=5618) 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=754) and cattle, including calves (N=265) were sent to the RIVM by the Animal Health Service concerning approximately 80% clinical Salmonella infections. Those from chickens (broilers, including poultry products, N=872; layers, reproduction animals and eggs, N=512) concerned mainly nonclinical Salmonella infections derived from a diversity of monitoring programs on the farm, slaughterhouses and at retail. In 2001, 2002, 2003 and 2004 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 – 2004 in the Netherlands.

	Total	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**
Human	5618	674	349	1056	862	1338	1339
Pig	754	31	195	114	168	127	119
Cattle	265	18	28	56	33	24	106
Chicken (misc.)	517	0	20	154	142	172	29
Broilers (faeces/meat)	872	68	100	164	238	192	110
Layers/Repro/Eggs	512	93	86	80	69	91	93
Other sources	1696	22	22	331	353	486	482
Total	10234	906	800	1955	1865	2430	2278

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

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

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

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

E. coli, E. faecium, E. faecalis and *Campylobacter* spp. isolated from slaughter pigs and broilers

E. coli and *E. faecium, E. faecalis* and *Campylobacter* spp. were isolated from faecal samples taken from healthy animals at slaughter by the National Inspection Service for Livestock and Meat (RVV). Six pig- and six broiler slaughterhouses respectively, were randomly selected. These slaughterhouses were situated all over the country to eliminate potential regional differences. The sampling period in 2004 was January - April. At each slaughterhouse once daily from one animal a faecal sample (pigs) was taken aseptically, or the caeca collected (broilers). The vials were stored at 4 – 8°C until the next Monday, when they were sent to CIDC-Lelystad. At the Department of Bacteriology and TSEs 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 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-KVW/RIVM. These samples were taken at farms from faeces of healthy animals. One isolate per farm was included. Isolates from non-human sources included strains isolated from samples taken in an attempt to trace a human infection.

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

Annually at the Animal Health Service large numbers of milk samples from clinical cases of bovine mastitis are sent in for bacteriological examination. From the isolates a selection of approximately 100 strains of *E. coli*, coliform bacteria, *S. aureus*, coagulase-negative staphylococci, *S. uberis* and *S. dysgalactiae* were sent to 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.

Brachyspira hyodysenteriae

Strains isolates by the Animal Health Service in Deventer from intestines of diseased animals and identified as *B. hyodysenteriae* were sent to CIDC Lelystad for susceptibility testing.

Mycoplasma synoviae

Mycoplasme strains isolated from diseased poultry were sent by the Animal Health Service in Deventer to CIDC Lelystad for susceptibility testing. The species identification was confirmed by PCR.

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 table 34.
B. hyodysenteriae was tested by broth dilution as described by Märit Pringle et al. in 2002. Mycoplasma's are by nature fastidious organisms and routinely not tested for susceptibility. Moreover validated and well standardised methodologies are lacking. To test the *M. synovia* strains for susceptibility a method adopted from the one described by P.C. Hannan in Veterinary Research in 2000 was used. As growth media ME-liquid medium (Mycoplasma experience Ltd) was used for the broth micro dilution test and as solid medium M.E. solid medium for avian mycoplasma's was used produced by the same company.
To determine the concentration of the inocula, for each strain serial dilutions of pure cultures were inoculated in ME-broth. Subsequently, for all five antibiotics used twofold dilutions concentration ranges varying from 0.03 – 64 µg/ml were prepared in microtitre trays I ME-broth and stored at -80°C pending analysis. To validate the concentration ranges of the antibiotics made and the potential effect of the broth and the incubation conditions (5% CO2, 37°C), ATCC control strains E. coli 25922 and S. aureus 29213 were inoculated in the test plates used. The results for the control strains (table 33) demonstrate that the results always complied with CLSI criteria and that the growth medium and incubation conditions have had no effect on the activity of the antibiotics in the microtitre trays.

Table 33. Results of QC-strains tested for susceptibility in the microtitre trays used for Mycoplasma synoviae.

Control strain	Antibiotic	MIC CIDC (µg/ml)	CLSI range (µg/ml)
E. coli ATCC 25922	Enrofloxacin	0.015 – 0.03	0.008 – 0.003
	Difloxacin	0.06 – 0.125	0.015 – 0.125
	Doxycycline	1	0.5 – 2*
	Tylosin	> 32	> 32
	Tilmicosin	> 32	≥ 64
S. aureus ATCC 29213	Enrofloxacin	0.125	0.03 – 0.125
	Difloxacin	0.25	0.06 – 0.5
	Doxycycline	0.125	0.125 – 0.5*
	Tylosin	1	0.5 – 4
	Tilmicosin	2	1 - 4
	Antibiotic	**MIC CIDC (µg/ml)**	**MIC Hannan et al. 2000 (µg/ml)**
M. synoviae ATCC 25204	Doxycycline	0.125	0.1#
	Enrofloxacin	0.5	0.5
	Tylosin	≤ 0.015	0.01

* CLSI control range

\# Hannan determined tetracycline MICs

The microtitre trays were inoculated with 50 µl of 10^4 Colour Changing Units/ml in each well. The plates were visually controlled for colour changes from red to yellow on days 1, 2, 3, 4, 7, 8, 9 and 14. From day 7 no further change in MIC was recorded, therefore the MIC recorded on day 7 was considered to be the accurate value.

Table 34. MIC-breakpoints (µg/ml) used for susceptibility testing of bacteria. Isolates with MIC-values higher than those presented in this table are considered resistant.

	Salmonella spp. *E. coli*	*Campylobacter* spp.	*Enterococcus* spp.	*Mycoplasma synoviae*	*E. coli (mastitis)*	*Streptococcus* spp.	*S. aureus.*	Coag. neg staphylococc	*Brachyspira* spp.
Penicillin	-	-	-	-	-	2	0,125	0,125	-
Oxacillin	-	-	-	-	-	-	2	0,25	-
Amoxicillin	16	16	8	-	16	-	-	-	-
Amox/clav. acid	16/8	-	8/4	-	16/8	8/4	4/2	4/2	-
Cephalothin	-	-	-	-	-	16	16	16	-
Cefuroxime	-	-	-	-	16	-	-	-	-
Cefoperazone	-	-	-	-	32	-	-	-	-
Ceftiofur	-	-	-	-	-	-	-	-	-
Cefquinome	-	-	-	-	4	-	-	-	-
Cefotaxime	1	-	-	-	-	-	-	-	-
Imipenem	1	-	-	-	-	-	-	-	-
Streptomycin	-	8	2000	-	32	-	16	16	-
Gentamicin	8	8	500		8	-	-	-	-
Kanamycin	-	-	-	-	16	-	16	16	-
Neomycin	16	8	-	-	16	-	16	16	-
Spectinomycin	-	-	-	-	-	-	-	-	-
Tetracycline	8	-	-	-	8	4	8	8	-
Doxycycline	4	4	8	8	-	-	-	-	-
Sulphamethoxazole	256	256	-	-	-	-	-	-	-
Trimethoprim	8	-	-	-	-	-	-	-	-
Trim/sulphamethoxazole	2/38	8/152	-	-	2/38	2/38	2/38	2/38	-
Nalidixic acid	16	16	-	-	-	-	-	-	-
Difloxacin	-	-	-	2	-	-	-	-	-
Enrofloxacin	-	-	-	1	2	-	-	-	-
Ciprofloxacin	2	2	8	-	-	-	-	-	-
Chloramphenicol	16	16	16	-	-	-	-	-	-
Florfenicol	16	-	-	-	-	-	-	-	-
Nitrofurantoine	-	-	128	-	-	-	-	-	-
Vancomycin	-	-	16	-	-	-	-	-	-
Teicoplanin	-	-	16	-	-	-	-	-	-
Avilamycin	-	-	16	-	-	-	-	-	-
Bacitracin	-	-	128	-	-	-	-	-	-
Flavomycin	-	-	16	-	-	-	-	-	-
Quinu/dalfopristin	-	-	2	-	-	-	-	-	-
Virginiamycin	-	-	8	-	-	-	-	-	-
Erythromycin	-	16	4	-	-	0,5	4	4	-
Tylosin	-	-	-	8	-	-	-	-	16
Tilmicosin	-	-	32	16	-	-	-	-	-
Lincomycin	-	-	-	-	-	4	4	4	-
Pirlimycin	-	-	-	-	-	2	2	2	-
Tiamulin	-	-	-	-	-	-	-	-	1
Metronidazole	-	4	-	-	-	-	-	-	-
Salinomycin	-	-	4	-	-	-	-	-	-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