MARAN 2008

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-2008 by VANTURES, the Veterinary Antibiotic Usage and Resistance Surveillance Working Group. The information presented in MARAN-2008 is based on a collation of data from ongoing surveillance systems on the use of antimicrobial agents in animal husbandry and the development of antimicrobial resistance in bacteria of animal origin and of relevance to public health.
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Summary, Conclusions and Recommendations

Usage of antibiotics

The extent to which antibiotics are used for veterinary purposes in food producing animals can contribute to public and animal health risks. It is an important determinant for the development of antibiotic resistance within the treated animal populations.

The main objective of this study is to obtain detailed insight into the exposure of farm animals to antibiotics in the Netherlands, by monitoring both at the national level and more specifically per animal species. This report provides an analysis of total sales figures, insight in the trend in use per animal species and also an international comparison. The results can be used by the Ministry of Agriculture, Nature and Food Quality, that commissioned the study, to provide information about antibiotic use to the European Commission. In addition, the usage data can play an important role in explaining trends in resistance that have become apparent. Moreover, trends in antibiotic use can be used to measure the effect of policy.

Trends in total sales of antibiotics in the Netherlands

Antibiotic usage on prescription expressed in terms of grams per kg live weight has doubled in 2007 compared to 1999, but has decreased in 2008. Recent figures reveal that in 2009 there has been a further slight decrease. During this same period, the antimicrobial growth promoters have been banned, first partly and as from 2006 entirely.

Tendencies in exposure to antibiotics in the Netherlands

The figures on exposure to antibiotics in the Netherlands expressed in terms of daily dosages per animal per year in the samples reveal the following tendencies for the years 2004 to 2008:

- sow/piglet farms: annual variation with constant usage from 2007 to 2008;
- fattening pig farms: increased usage from the year 2005;
- broiler farms: increased usage from 2004;
- veal calf farms: decreased usage from 2007 to 2008;
- dairy farms: annual variation with increased usage from 2006.

The usage in fattening pigs in 2008 is statistically significantly higher than the use in 2005. Also the usage in broilers in 2008 is statistically significantly higher than the use in 2005.

Trends in the total sales of antibiotics in European countries

The comparison of several European countries for which figures about veterinary antibiotics are available shows that the sales in 2008, expressed in grams per kg live weight, does not differ much from 2007 in most countries, whereas there has been a decrease of more than 5% in Norway, France and the Netherlands.

Trends in resistance

In 2008, 65 cefotaxime resistant *Salmonella* isolates were found, which is indicative of production of extended spectrum beta-lactamases (ESBLs). The isolates belonged predominantly to the serovar *S*. Java (69%, N = 45) all of which were isolated from poultry sources. Other ESBL-suspected serovars were Agona, Infantis, Senftenberg, Typhimurium Ft90, Enteritidis PT 4, Virchow, Kottbus, Senftenberg, Cubana, Rissen and Heidelberg, all from poultry; Stanley, Typhimurium Ft 80, FT 510, Montevideo, *S. enterica* subspecies *enterica* 1,4,5,12:i:- and Derby from humans, and Dublin from cattle. Up to now, poultry is the only reservoir for ESBL-producing salmonella's in animals, which is associated with the transmission of the genetic determinant between *Salmonella* and ESBL-producing *E. coli* in poultry. Third-generation cephalosporins are not used in broiler production, but the use of ceftiofur in combination with Marek vaccine or with in ovo vaccination is a common off-label use procedure in the poultry reproduction and breeding sectors. It is likely that this has contributed to selection and vertical transmission of ESBLs in the poultry production pyramid.

In 2008 in *S.* Typhimurium isolates from human infections, a very striking increase was observed in ciprofloxacin resistance (31.3%) compared to 3.7% in 2006/2007. This increase was solely due to DT104, of which 14% were isolated from various animal sources and 86% from human infections. This is a worrisome development since DT104 is associated with increased virulence. It can be expected that human infections with ciprofloxacin reduced susceptible, nalidixic acid resistant strains cause more complications and treatment failure. The source or sources for this increased incidence is unknown, but not likely a Dutch animal source, because these isolates occur still rather infrequently in Dutch food-producing animals. The involved strains concerned a food born outbreak at the end of 2008 of which the MLVA pattern points to quinolone resistant DT104 strains involved in outbreaks abroad.

S. Java was in 2008 still the most predominant serovar isolated in broiler production. This is confirmed by the isolation rate of 76% of this serovar from poultry products in 2008. The *S.* Java isolates from poultry are clonally related and totally adapted to poultry, rarely causing human infection. The clone is typically resistant against trimethoprim and

streptomycin (low level), but has in addition acquired many other resistance determinants: ESBLs, tetracycline resistance genes and chromosomal and plasmid mediated quinolone resistance.

In *Campylobacter* resistance against the fluoroquinolones continues to increase in 2008 in isolates from animals and from humans. In 2008 approximately 50% of the *Campylobacter* isolates from humans were resistant against ciprofloxacin, compared to 35% in the period 2002-2005. More than 60% of the isolates from broiler chickens were ciprofloxacin resistant compared to 35-45% in 2002 - 2005.
In general, *C. coli* showed much more resistance and at higher MIC levels than *C. jejuni*.
Macrolide resistance remains low in *C. jejuni*. In 2008, 5.6% of the *C. jejuni* strains were resistant against erythromycin. Five *C. jejuni* strains were detected with an MIC of 128 mg/L for erythromycin, of which four were from broiler chicken faeces and one strain was isolated from a veal calf.

Resistance rates in *E. coli* continue to increase in slaughter pigs, broiler chickens and dairy cows. Also in veal calves resistance is high, but for most antibiotics that were tested, rates seem either to stabilize or to show a moderate decrease.
In dairy cows, resistance in *E. coli* has been traditionally low, but is increasing alarmingly fast in a few years time. In 2005, multidrug resistance was rarely observed, while in 2008 11% of the isolates were resistant to two or more antibiotic classes and resistance against up to eight classes was seen in individual isolates.
In broiler chickens, resistance against the quinolones is again disturbingly high and moreover still increasing. In 2008, more than 60% of all *E. coli* isolates were resistant against nalidixic acid and reduced susceptible for ciprofloxacin of which 6.3% were high level ciprofloxacin resistant (MICs 1 - >8 mg/L).
Another matter of concern is the emergence of extended spectrum beta-lactamases (ESBL). ESBLs are detected in all *E.coli* of food-producing animals at low levels. The increase observed since 2003 in isolates from broiler chickens is alarming. In 2008, approximately 15% of the randomly isolated *E. coli* from chickens and chicken meat products were resistant against cefotaxime and ceftazidime, indicative of the frequent presence of ESBL's. In a recent prevalence study on 26 broiler farms it was determined that 100 % of investigated farms were positive for ESBL-producing *E. coli* and that on 85% of these farms ≥80% (95% CI 71-99%) of the animals carried ESBL-producers in their faeces.

For both *E. faecalis* and *E. faecium*, high resistance levels were observed for tetracycline, erythromycin and streptomycin. In addition, in *E. faecium* resistance rates were high for quinu/dalfopristin (73.8% vs. 1.9% in *E. faecalis*) and salinomycin (40% vs. 10.3% in *E. faecalis*). Ampicillin resistance was only observed in *E. faecium*. No resistance was observed against linezolid and florfenicol.
Compared to previous years, the number of high level ciprofloxacin resistant *E. faecalis* and *E. faecium* isolates (MIC ≥16 mg/l) in 2008 have increased.
Vancomycin resistance was observed in *E. faecium* strains isolated from all animal species included in this survey, although at a very low level.

MRSA isolated from Dutch food producing animals were included in the surveillance in order to assess animal or human health risks. To determine the occurrence of additional resistance characteristics next to those associated with the *mecA*-gene are important because of the zoonotic potential of animal related MRSA.
Most MRSA isolates (97%) were tetracycline resistant, and all belonged to ST398. Most tetracycline susceptible isolates belonged to MLST types other than type 398. In addition, more than 60% of the MRSA isolates were resistant against erythromycin and clindamycin. Compared to previous findings in pig isolates these data reflect an increase in resistance against lincosamides. This is an important trend which affects the preference of antibiotics for therapeutic treatment in human patients. In hospital settings, clindamycin has been advised as empirical treatment for animal related MRSA infections.
Ciprofloxacin resistance was commonly found, being highest in MRSA from poultry.
Resistance against the aminoglycosides (gentamicin and neomycin) showed considerable variation (from 15 to 57%), highest levels of resistance were found in isolates from veal calves. Resistance against the drug combination trimethoprim/sulphamethoxazole was rarely detected.
Importantly, animal MRSA isolates showed no resistance against vancomycine or mupirocin, and sporadic reduced susceptibility to fusidic acid and rifampin. These four antibiotics are considered important drugs for the treatment and decontamination of MRSA in hospital settings.
Multi drug resistance was wide spread in animal MRSA. Multi drug resistance was generally found against beta-lactam antibiotics, macrolides, lincosamides, aminoglycosides (neomycin and gentamicin) and fluoroquinolones.

Regarding the animal pathogens, resistance levels were low in both *Pasteurella multocida* and *Mannheimia haemolytica* strains from cattle with respiratory disease. With the exception of tetracycline resistance in *Mannheimia*, resistance levels were all below 10%, which is regarded as the threshold for the empirical use of antibiotics.

Resistance levels in *E. coli* strains isolated from mastitis milk samples from dairy cows were generally low to moderate. Highest levels were observed against tetracycline, streptomycin and ampicillin. As in previous years, ESBL

producing *E. coli* strains were isolated from milk samples from dairy cows. In comparison, the coliform bacteria showed a higher level of resistance against ampicillin (85%) and amoxicillin-clavulanic acid (22%).
Staphylococcus aureus strains had low levels of resistance against most antibiotics. Again, a methicillin-resistant *S. aureus* (MRSA) was isolated from a milk sample from a cow with mastitis.
In general, coagulase negative staphylococci were more resistant than *S. aureus*. In 2008, 53% were penicillin resistant and 3% oxacillin resistant (*mecA* positive). Based on epidemiological cut off values, 24% of the coagulase negative staphylococci were reduced susceptible with regard to clindamycin, compared to 1% of the *S. aureus* isolates.

Conclusions and recommendations
After a period of continuous increase of on prescription usage of antibiotics in food animals in The Netherlands for the first time in 2008 the total sales of antibiotics per kg live weight has decreased. An international comparison shows that in most investigated European countries the sales in 2008 does not differ much from 2007, whereas there has been a decrease of more than 5% in Norway, France and the Netherlands. Sample data about the use in specific animal species in the Netherlands reveals a varied situation: tendencies to a further increase in fattening pigs, broilers and dairy cattle, constant usage in sows/piglets and a decrease in veal calves.

In 2008, the levels of antimicrobial resistance in bacteria from the major food-producing animal species in The Netherlands, cattle, slaughter pigs and broilers, were comparable to former years. Exceptions were ciprofloxacin resistance in indicator *E. coli* from broilers and *Campylobacter* from broilers and humans, which showed an increase in occurrence.
Like in previous years in broiler chickens highest resistance levels were observed. This indicates that in broiler production optimum circumstances exist for selection and dissemination of resistant bacteria. In these animals Extended Spectrum Beta-Lactamase (ESBLs) producing *E. coli* occur frequently in the faeces and on poultry meat products. Moreover, evidence for transmission of ESBLs in poultry to *Salmonella* exists. Also in all other animal species included in the surveillance ESBL-producing *E. coli* isolates were observed, but at low levels
Animal associated MRSA isolates have acquired several additional resistance traits, which makes them highly multidrug resistant. However, resistance against the most important drugs in health care is still absent.
The data demonstrate that multi drug resistant isolates with a public health concern like MRSA and ESBL-producers are common on food animal production. To understand the current and future evolution of public health implications of these organisms, the surveillance should be targeted towards the molecular aspects of the organisms.

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

- A detailed and independent monitoring of the veterinary use of antibiotics remains important to provide an adequate insight into the true exposure on the level of animal species. Insight into the exposure is necessary to relate the usage data to the development of antimicrobial resistance.
- In the next few years EU member states have to develop a similar and uniform monitoring, at first based on national sales data. Furthermore, within the EU also an additional monitoring per animal species needs to be pursued.
- Continuous surveillance of molecular characteristics of isolates of public health concern (ESBL-producers and MRSA) is necessary to understand the current and future public health hazard related to these organisms. This should preferably be conducted in close collaboration with the medical sectors.

Samenvatting, Conclusies en Aanbevelingen

Gebruik van antibiotica
De mate waarin antibiotica worden gebruikt voor therapeutische doeleinden bij voedselproducerende dieren kan bijdragen aan de volksgezondheid en de diergezondheid risico's. Het is een belangrijke determinant voor de ontwikkeling van resistentie tegen antibiotica in de behandelde dierpopulaties.
Het belangrijkste doel van deze studie is om gedetailleerd inzicht te krijgen in de blootstelling van landbouwhuisdieren aan antibiotica in Nederland, door monitoring zowel op nationaal niveau en meer specifiek per diersoort. Dit rapport geeft een analyse van de totale verkoop cijfers, inzicht in de trends in gebruik per diersoort en ook een internationale vergelijking. De resultaten kunnen worden gebruikt door het Ministerie van Landbouw, Natuur en Voedselkwaliteit, de opdrachtgever van deze studie, voor het verstrekken van informatie over het gebruik van antibiotica in Nederlandse dieren aan de Europese Commissie. Bovendien kunnen deze gegevens een belangrijke rol spelen in het verklaren van trends in de resistenties in bacteriën uit landbouwhuisdieren. Bovendien kunnen trends in gebruik van antibiotica worden gebruikt voor het meten van het effect van het beleid.

Trends in de totale verkoop van antibiotica in Nederland
Het gebruik van antibiotica op voorschrift van een dierenarts uitgedrukt in gram per kg levend gewicht is verdubbeld in 2007 vergeleken met 1999, maar is gedaald in 2008. Uit recente cijfers blijkt dat in 2009 er een verdere lichte daling is geweest. In dezelfde periode zijn de antimicrobiële groeibevorderaars eerst gedeeltelijk verboden en vanaf 2006 volledig.

Tendensen in de blootstelling aan antibiotica in Nederland
De cijfers over blootstelling van dieren aan antibiotica in Nederland, uitgedrukt in dagdoseringen per dierjaar geven de volgende tendensen te zien in de jaren 2004 tot 2008:
- zeugen/biggen bedrijven: jaarlijkse variatie met gelijkblijvend gebruik in 2007-2008;
- vleesvarkensbedrijven: toename in gebruik na 2005;
- vleeskuikenbedrijven: toename in gebruik na 2004;
- vleeskalverbedrijven: afname in gebruik van 2007-2008;
- melkveebedrijven: jaarlijkse variatie met een toename in gebruik na 2006.

Het gebruik bij vleesvarkens is in 2008 statistisch significant hoger dan het gebruik in 2005. Ook het gebruik bij vleeskuikens is in 2008 statistisch significant hoger dan het gebruik in 2005.

Trends in de totale verkoop van antibiotica in de Europese landen
De vergelijking van een aantal Europese landen waarvoor cijfers over de veterinaire antibiotica beschikbaar zijn laat zien dat in de meeste landen de verkoop in 2008, uitgedrukt in gram per kg levend gewicht, niet veel verschilt met 2007, terwijl er een daling van meer dan 5% is in Noorwegen, Frankrijk en Nederland.

Trends in resistentie
In 2008 werden 65 cefotaxime resistente *Salmonella* isolaten gevonden, wat indiceert dat deze isolaten extended spectrum beta-lactamasen (ESBLs) produceren. Deze isolaten behoorden voornamelijk tot serotype *S.* Java (69%, N = 45), die allen werden geïsoleerd uit pluimvee. Andere ESBL-verdachte serotypen waren Agona, Infantis, Senftenberg, Typhimurium Ft90, Enteritidis PT 4, Virchow, Kottbus, Senftenberg, Cubana, Rissen and Heidelberg, allemaal uit pluimvee; Stanley, Typhimurium Ft 80, FT 510, Montevideo, *S. enterica* subspecies *enterica* 1,4,5,12:i:- en Derby uit mensen, en Dublin uit een rund. Tot nu toe is pluimvee het enige reservoir voor ESBL producerende salmonella's in dieren. Dit is geassocieerd met transmissie van het ESBL-gen tussen *Salmonella* en ESBL producerende *E. coli* in pluimvee. Derde generatie cefalosporinen worden in vleeskuikens niet gebruikt, maar het gebruik van ceftiofur in combinatie met Marek vaccin of met in ovo vaccinatie is een vorm van off-label gebruik in de pluimvee reproductie en fokkerij sectoren. Het is waarschijnlijk dat deze vorm van gebruik heeft bijgedragen aan de selectie en verspreiding van ESBLs in the pluimvee productie piramide.

In 2008 in *S.* Typhimurium isolaten uit mensen, werd een opvallende toename gezien in ciprofloxacin resistentie (31.3%) in vergelijking met 3.7% in 2006/2007. Deze toename werd alleen veroorzaakt door faagtype DT104, waarvan 14% werden geïsoleerd uit verschillende dierlijke bronnen en 86% uit mensen met infecties. Dit is een opvallende toename omdat DT104 is geassocieerd met virulentie. Het valt te verwachten dat humane infecties met ciprofloxacin verminderd gevoelige stammen meer complicaties geven en moeilijker te behandelen zijn. De bron van deze verhoogde incidentie is waarschijnlijk niet afkomstig uit Nederlandse bron daar we dergelijke isolaten in Nederlandse voedsel producerende dieren slechts zelden tegenkomen. Het betrof hier een cluster van voedselinfecties, eind 2008, met *Salmonella* stammen waarvan het MLVA patroon wijst op quinolonen resistente DT104 stammen die ook explosies van infecties veroorzaakten in het buitenland.

In 2008 werd *S.* Java nog steeds het meest geïsoleerd in vleeskuikens en vleeskuiken producten. De *S.* Java isolaten uit pluimvee behoren tot een kloon die geheel aangepast is aan kippen en slechts zelden de mens infecteert. De kloon is altijd resistent tegen trimethoprim en streptomycine (low level), maar heeft daarnaast allerlei additionele resistenties verworven, zoals ESBLs, tetracycline resistentie genen en chromosomale en via plasmiden overdraagbare quinolonen resistentie.

In *Campylobacter* neemt in 2008 resistentie tegen fluoroquinolonen nog steeds toe zowel in isolaten uit dieren als mensen. In 2008 waren ongeveer 50% van de *Campylobacter* isolaten uit mensen met diarree resistent tegen ciprofloxacin, terwijl dit in de periode 2002-2005 rond de 35% lag. Meer dan 60% van de isolaten uit vleeskuikens waren in 2008 ciprofloxacin resistent, terwijl dit 35-45% was in 2002 - 2005.
In het algemeen vertoonde *C. coli* veel meer resistenties en ook hogere MIC niveaus dan *C. jejuni*.
Macrolide resistentie blijft laag in *C. jejuni*. In 2008, waren 5.6% van de *C. jejuni* isolaten resistent tegen erythromycine. Er werden 5 *C. jejuni* isolaten gevonden met een hoge MIC van 128 mg/L voor erythromycine, waarvan er vier werden geïsoleerd uit vleeskuikens en één uit een vleeskalf.

De resistentieniveaus in *E. coli* nemen nog steeds toe in vleesvarkens, vleeskuikens en melkkoeien. Hoewel ook in vleeskalveren de resistentieniveaus in 2008 hoog zijn, lijken deze zich voor de meeste antibiotica te stabiliseren of een geringe afname te vertonen.
In melkkoeien kwam resistentie in de darmflora eigenlijk maar zelden voor. Het is dan ook opvallend dat in slechts enkele jaren een duidelijke toename wordt gezien. In 2005 werd bij melkkoeien multiresistentie slechts zelden waargenomen, terwijl in 2008 11% van de *E. coli* isolaten resistent waren tegen twee of meer antibiotica klassen en resistentie tegen maximaal acht klassen werd gezien in individuele isolaten.
In vleeskuikens is het resistentieniveau voor de quinolonen nog steeds erg hoog en het lijkt bovendien nog steeds toe te nemen. In 2008, waren meer dan 60% van alle isolaten resistent tegen nalidixinezuur en verminderd gevoelig voor ciprofloxacin, 6.2% hiervan vertoonde klinische ciprofloxacin resistentie.
Het voorkomen van ESBL producerende *E. coli* isolaten is een andere reden van zorg. Deze worden in alle voedselproducerende dieren op een laag niveau gezien. Echter, de toename van cefalosporinen- resistente *E. coli* in de darmflora van vleeskuikens is alarmerend. In 2008 waren ongeveer 15% van de aselect verzamelde *E. coli* isolaten uit kuikens and pluimveevlees resistent tegen cefotaxime and ceftazidime, wat indicatief is dat veel vleeskuikens in hun darmkanaal drager zijn van ESBL-producerende stammen. . In een recent uitgevoerde prevalentie studie op 26 vleeskuikenbedrijven werd vastgesteld dat 100% van de onderzochte bedrijven positief was voor ESBL-producerende *E. coli* en op 85% van deze bedrijven ≥80% (95% CI 71-99%) van de dieren ESBL-producerende bacteriën in hun darminhoud hebben.

Voor zowel *E. faecalis* als *E. faecium*, werden hoge niveaus van resistentie gezien voor tetracycline, erythromycine en streptomycine. Daarnaast was *E. faecium* in hoge mate resistent tegen quinu/dalfopristin (73.8% vs. 1.9% in *E. faecalis*) en salinomycine (40% vs. 10.3% in *E. faecalis*). Ampicilline resistentie werd alleen gezien in *E. faecium* en er werd geen resistentie gezien tegen linezolid en florfenicol.
In vergelijking met eerdere jaren is het aantal ciprofloxacin resistente *E. faecalis* en *E. faecium* isolaten (MIC ≥ 16 mg/L) in 2008 toegenomen.
Nog steeds werden in alle diersoorten in de surveillance enkele vancomycine resistente *E. faecium* isolaten gezien.

MRSA uit Nederlandse voedselproducerende dieren werden onderzocht in de surveillance om een indruk te krijgen van eventuele dier-, en volksgezondheidsrisico's. Het vaststellen van de aanwezigheid van additionele resistentiegenen naast het *mec*A-gen is van belang in verband met de zoönotische eigenschappen van diergerelateerde MRSA.
De meeste MRSA isolaten (97%) waren tetracycline resistent (allen ST398). De meeste tetracycline gevoelige isolaten behoorden tot andere MLST typen dan ST 398. Meer dan 60% van de MRSA isolaten waren ook resistent tegen erythromycine en clindamycine. Dat is een toename in vergelijking met eerdere bevindingen in MRSA uit varkens. Dit is van belang omdat deze trends de eerste keuze therapie in de gezondheidszorg beïnvloedt. In ziekenhuizen is geadviseerd om clindamycine als eerste keuze middel te gebruiken voor empirische behandeling van infecties door diergerelateerde MRSA. Ciprofloxacin resistentie werd vaak gezien, vooral in isolaten uit pluimvee.
Resistentie tegen de aminoglycosiden (gentamicine en neomycine) vertoonde aanzienlijke variatie (van 15 to 57%), de hoogste waarden werden gevonden in isolaten uit vleeskalveren. Resistentie tegen de combinatie trimethoprim/sulphamethoxazole werd slechts een enkele keer gezien.
Het is van belang dat in diergerelateerde MRSA geen resistentie tegen vancomycine en mupirocine werd gevonden en dat slecht incidenteel verminderde gevoeligheid voor fusidinezuur en rifampicine werd gezien. .Deze vier antibiotica worden als belangrijk voor behandeling en decontaminatie van MRSA in de gezondheidszorg gezien. Multiresistentie kwam erg veel voor in diergerelateerde MRSA, vooral tegen beta-lactam antibiotica, macrolides, lincosamiden, aminoglycosiden (neomycine en gentamicine) en fluoroquinolonen.

Voor de luchtwegpathogenen *Pasteurella multocida* en *Mannheimia haemolytica* uit rundvee werden in het algemeen lage resistentieniveaus gezien. Met uitzondering van tetracycline resistentie in *Mannheimia*, waren de warden lager dan 10%, wat als drempelwaarde wordt beschouwd voor empirische eerste keuze therapie.

Ook de resistentieniveaus in *E. coli* isolaten uit mastitis melkmonsters van koeien waren in het algemeen laag tot gemiddeld. De hoogste waarden werden gezien voor tetracycline, streptomycine en ampicilline. Net als in voorgaande jaren werden enkele ESBL producerende *E. coli* isolaten gevonden in melkmonsters van koeien. In vergelijking met *E. coli* vertoonden de coliforme uierbacteriën hogere resistentieniveaus voor ampicilline (85%) and amoxicilline-clavulaanzuur (22%).
In *Staphylococcus aureus* uit mastitis melk werden tegen de meeste antibiotica lage resistentiewaarden gevonden. Net als in 2006/2007 was één van de 101 onderzochte *S. aureus* isolaten uit mastitis melk een methicilline-resistente *S. aureus* (MRSA).
In het algemeen waren coagulase negatieve staphylokokken vaker resistent dan *S. aureus*. In 2008 waren 53% resistent tegen penicilline en 3% tegen oxacilline (*Mec*A positief). Daarnaast waren 24% van de coagulase negatieve staphylokokken verminderd gevoelig voor clindamycine, in vergelijking met 1% resistentie in *S. aureus*.

Conclusies en aanbevelingen
Na een periode van voortdurende toename van het antibioticagebruik bij landbouwhuisdieren in Nederland op voorschrift van een dierenarts, is de totale hoeveelheid verkochte antibiotica, uitgedrukt per kg levend gewicht, in 2008 voor het eerst gedaald. Uit een internationale vergelijking blijkt dat in de meeste onderzochte Europese landen de verkopen in 2008 weinig anders zijn dan die in 2007, terwijl er een afname van meer dan 5% is geweest in Noorwegen, Frankrijk en Nederland. Steekproefgegevens over het antibioticagebruik in specifieke diersoorten laten een gevarieerd beeld zien: een tendens tot een verdere toename van het gebruik bij vleesvarkens, vleeskuikens en melkvee, een gelijkblijvend gebruik bij zeugen/biggen en een afname bij vleeskalveren.

De niveaus van antimicrobiële resistentie in bacteriën uit de belangrijkste Nederlandse voedselproducerende dieren, runderen, varkens en vleeskuikens, waren relatief stabiel in 2008. Uitzonderingen waren ciprofloxacin resistentie bij *E. coli* als indicatorbacterie van vleeskuikens en *Campylobacter* van vleeskuikens en de mens, waar een toename gezien werd.
Net als in voorgaande jaren werden in vleeskuikens de hoogste resistentieniveaus waargenomen. Dit geeft aan dat in vleeskuikens optimale omstandigheden aanwezig zijn voor de selectie en de verspreiding van resistente bacteriën. In deze dieren komen Extended Spectrum Beta-lactamase (ESBL's) producerende *E. coli* vaak voor in de ontlasting en op pluimveevlees. Bovendien komt in pluimvee overdracht van ESBL's naar Salmonella voor. Ook in alle andere diersoorten werden ESBL-producerende *E. coli* isolaten werden waargenomen, maar op een laag niveau.
Diergerelateerde MRSA-isolaten hebben diverse extra resistentiegenen verworven, waardoor ze zeer multiresistent zijn geworden. Resistentie tegen de belangrijkste middelen in de gezondheidszorg is nog steeds afwezig.
De gegevens tonen aan dat multiresistente isolaten met een potentieel volksgezondheidsrisico, zoals MRSA en ESBL-producenten, veel voorkomen in landbouwhuisdieren en vleesproducten. Voor het begrijpen van de huidige en toekomstige ontwikkelingen op het gebied van de volksgezondheidsrisico's van deze organismen moet de surveillance worden gericht op de moleculaire aspecten van de organismen.

Gebaseerd op de gegevens in dit verslag kan worden aanbevolen dat:

- Een gedetailleerde en onafhankelijke monitoring van het veterinair gebruik van antibiotica blijft van belang voor een goed inzicht in de daadwerkelijke blootstelling op diersoortniveau. Inzicht in de blootstelling is noodzakelijk om de relatie te kunnen leggen met de ontwikkeling van resistentie.
- In de lidstaten van de EU zal in de komende jaren een vergelijkbare en uniforme monitoring moeten worden opgezet, in eerste instantie gebaseerd op landelijke verkoopcijfers. Binnen de EU zal tevens een uitbreiding van deze monitoring naar gegevens over de blootstelling op diersoortniveau moeten worden nagestreefd.
- Continue bewaking van de moleculaire kenmerken van isolaten met een potentieel volksgezondheidsrisico (ESBL-producenten en MRSA) is noodzakelijk om de huidige en toekomstige risico's voor de volksgezondheid te kunnen beoordelen. Dit moet bij voorkeur worden uitgevoerd in nauwe samenwerking met de medische sector.

I Usage of antibiotics in animal husbandry in the Netherlands

1. Introduction

Problem definition
Previous MARAN reports have revealed that although the total number of animals produced in the Netherlands steadily decreased, the on prescription use of antibiotics increased until 2007. At first in 2008 a decrease was observed. The extent to which antibiotics are used for veterinary purposes in food producing animals can contribute to public and animal health risks, because it is an important determinant for the development of antibiotic resistance**.** This is also recognised by the European Commission: all EU member states are required to monitor antimicrobial resistance in food producing animals of public health concern (Zoonosis Directive 2003/99/EC). Within this context, monitoring of antibiotic usage is equally important. A political mandate is provided by the European Commission (EC) to start collecting data on the usage of veterinary antibiotics. According to the EC Directive 2001/82/EC and Regulation 726/2004 there is a legal basis for national authorities to request the pharmaceutical industry to provide data on sales of antimicrobial agents. However, national authorities are not yet obliged to provide data about the use of veterinary antibiotics to the EC.

This report contains information about the usage monitoring results in the Netherlands, based on both sales data provided by the pharmaceutical industry and data on usage on sample farms.

Reasons for changes in use of antibiotics
Various developments in the Netherlands may have had a negative or positive impact on the veterinary usage of antibiotics during the last decade:

- the prohibition of the use of growth promoters as from 1999, leading to a final ban on January 1st, 2006;
- the prohibition on animal protein in feed;
- increase in farm sizes;
- the emergence of infectious diseases like PIA, PRRS and Circo-virus infections in pigs, chronic enteritis of unknown ethiology in poultry, respiratory and digestive disorders and dysbacteriosis in veal calves;
- increased awareness of the need to limit the use of antibiotics, as a result of the discovery of human patients infected with MRSA originating from livestock;
- increased concerns about the high and increasing antibiotic use both inside and outside the agricultural sector;
- the response of the sector and the authorities to those concerns by agreeing upon covenants designed to reduce antibiotic use.

Besides these developments also weather conditions (temperature, humidity etc.) may significantly influence animal health problems and consequently also influence the usage of antibiotic.

Monitoring of national sales data
Since 1998 FIDIN, a federation of the Dutch veterinary pharmaceutical industry, annually reports antibiotic sales figures in the Netherlands (FIDIN, 2009). These reports are produced on a voluntary basis**.** The sales figures stated in the reports give an impression of the total number of kilograms of antibiotics (active ingredients) used in the Netherlands at the level of pharmacotherapeutic groups (the groups of active ingredients, such as tetracyclines and quinolones).

Monitoring on a sample of farms
Besides monitoring of total sales data at the national level it is important to obtain insight into the real exposure of farm animals to antibiotics and also into the underlying factors that could explain changes in antibiotic use. For that reason there is also a more detailed monitoring of antibiotic use on a stratified sample of Dutch farms that supply data to the Farm Accountancy Data Network (FADN) of LEI Wageningen UR. In MARAN 2008 for the first time also data about veal calves are included, based on an additional aselect sample. This additional sample and the collection of data have been set up and executed in cooperation with the veal calf sector.

Objective and result
The main objective of this study is to obtain detailed insight into the exposure of farm animals to antibiotics, by monitoring both at the national level and more specifically per animal species. This report provides an analysis of total sales figures, nationally and an international comparison, and also insight in the trend in use per animal species.

Effect
The results from the study can be used by the Ministry of Agriculture, Nature and Food Quality to provide information about antibiotic use to the European Commission. In addition, the usage data can play an important role in explaining trends in resistance that have become apparent. Moreover, trends in antibiotic use can be used to measure the effect of policy.

2. Materials and methods

2.1 Analysis of sales data and country comparison

The FIDIN reports present the total number of kilograms of antibiotics (active ingredient) sold in the Netherlands at the level of pharmacotherapeutic groups**.** The data about use of active substances are based on sales data of members of FIDIN and are estimated to cover about 98% of all sales. Actual use can be different from the amounts sold as a result of stock piling and cross border use. The figures give insight in the total sales for all animals, not per individual animal species**.**

The total sales figures published by FIDIN have been related to the number and total live weight of animals in the Dutch livestock farming sector (pigs, broilers, veal calves, cattle, and sheep). This yields information about the trend in the sales of antibiotics in grams per kilogram of live animal weight over the years, thus taking yearly fluctuations in the size of the animal population into account.

The country comparisons are generally based on national sales data and Eurostat figures on animal numbers for different European countries. Note that there are differences in the level at which records are kept: in the Netherlands, France, Germany, UK, Finland and Norway at a national sales level and in Denmark and Sweden, as from 2003, at farm level (prescription level).

2.2 Farms in the Farm Accountancy Data Network

This report reviews the antibiotic usage in 2008, and is based on a total of 237 pig, broiler and dairy cattle farms in the Farm Accountancy Data Network. The results about veal calves are based on 186 farms in a large additional sample. See table 2.1 for details.

The Data Network contains a representative sample of around 1,500 agricultural and horticultural farms in the Netherlands (Vrolijk and Van der Veen, 2008). Records are made of the economic data and technical key figures of these farms. Every year a number of farms are replaced by other farms to ensure that the database of the Data Network remains representative for Dutch livestock farming. Detailed records have been kept of the animal-medicine data and veterinary services. The data for the veal calves originate from an aselect sample of the veal calf farms that were additionally collected. On these farms detailed data were collected on number of animals present and the detailed records on the amount of antibiotics used.

Detailed data are available on the websites of LEI (http://www.lei.wur.nl) and CVI (http://www.cvi.wur.nl/). The detailed data provide further insight into the use in daily dosages per administration method and about the use in grams of active ingredients per animal year. Note that the detailed data only apply to the farms in the sample(s). The data presented at a more aggregate level are considered to be representative for the total exposure to antibiotics at national level. All the same, the further details of these data cannot be directly interpreted as information representative for the details of the usage at national level, for example the use of a specific active substance.

Table 2.1 Number of animals and farms taking part each year and the associated number of animals

	Type of holding	2004	2005	2006	2007	2008
Number of animals	Sows/piglets	17467	16790	13642	19861	19079
	Fattening pigs	58617	58622	61503	128132	158210
	Broilers	801914	1961981	2047487	1930923	2563231
	Veal calves	n.a.	n.a.	n.a.	125125	131879
	Dairy cows	3929	2962	3099	3025	7273
Number of farms	Sows/piglets	49	46	34	42	47
	Fattening pigs	39	42	33	51	79
	Broilers	15	29	29	29	29
	Veal calves	n.a.	n.a.	n.a.	182	186
	Dairy cows	45	36	37	36	82
	Total	148	153	133	340	423

n.a. = no data available

2.3 Unit of measurement: defined daily doses per animal year (DDDanimal)

To provide insight in the true exposure of animals to antibiotics the use is expressed in the number of defined daily doses per animal year: DDDanimal.

Antibiotics vary in their potency and pharmacokinetic properties[1], and this is manifested in the form of varying dosages per kilogram of body weight between and within antibiotic classes. Because of the large variation in dosages of antibiotics the unit "grams per kg live weight", as calculated from total sales figures, is a less meaningful indicator for the use of antibiotics. The unit "daily dosage" is suitable for calculating the total exposure to different antibiotics and, for example, making comparisons per group. Adopting this approach offers an opportunity to obtain an improved insight into the relationship with the existence of or trends in the development of resistance. Moreover, this unit conforms to international developments in this field and developments in the human health sector. The broader implementation of records of this nature will also improve the feasibility of comparing the resultant data, for example the antibiotic use in different EU member states in similar livestock systems**.**

The number of daily dosages per animal year was determined by calculating the total number of kilograms of animal that can be treated with each active ingredient: the treatable weight. This was then divided by the total weight of the number of livestock on the farm[2]. This assumes that the average treatment is administered to animals with an average weight. With this approach the calculation and comparison of the total antibiotic use on farms is possible, even when different active ingredients are involved. Expressing the use per animal year provides for comparisons of farms with different vacancy periods. However, especially in some sectors (e.g. veal calves) differences in length of production periods should also be taken into account**.**

This information can then be used to obtain an insight into the total antibiotic use for specific animal species and categories of animal species (for example, fattening pigs) on a specific group of farms (for example, all pig farms with fattening pigs). This is expressed in terms of an average number of daily dosages per animal year for fattening pigs**.** More information about this unit of measurement is given in the following daily dosages box, which also includes an example of a calculation.

Animal weights

In general younger animals are more likely to encounter health problems than older animals, while animals no longer receive antibiotics in the last period before slaughter, primarily because of less health problems and also to ensure that the meat is free of antibiotic residues**.** The best estimation of the total treatment duration per year would be obtained by calculating the number of daily dosages on the basis of the best possible estimate of the average weight *on treatment*. However, the information currently available is not sufficient to determine the exact weight of the animals at the time of the administration of the medicine. For this reason the calculations in this report are still based on the average weight per animal during the animals' presence on the farm. The calculated number of defined daily dosages is therefore expected to be an underestimation of the actual exposure, especially for piglets, fattening pigs and veal calves.

The following average weights have been used: dairy cow 600 kg, young calves (birth to weaning) 56.5 kg, white veal calf 164 kg, rosé veal calf 205 kg, broiler 1.0 kg, fattening pig 70.2 kg, sow 220 kg, maiden gilt 107.5 kg, piglet 12.5 kg, breeding boar 350 kg (ASG, 2009). For sow farms, the weight of the average number of sows, gilts, piglets and breeding boars is totalled.

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

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

Daily dosages
The amounts of different active ingredients cannot simply be totalled since the antimicrobial potency and pharmacokinetics (and, consequently, the dosage prescription) varies between active ingredients**.** However, active ingredients can be compared and totalled once the active ingredient in each antibiotic preparation is expressed in terms of the *daily dosage*. The daily dosage is a measure of the number of milligrams of a specific active ingredient required to treat one kilogram of animal in one day with that antibiotic preparation, and is based on the recorded average dosage of a medicine for a specific type of animal[3]. These daily dosages can be totalled to determine the total exposure to antibiotics**.** The daily dosages are specific to the type of animal, and have been defined for dairy cattle, veal calves, pigs and poultry. Consequently, antibiotic preparations may have been assigned multiple daily dosages, according to the type of animal the preparations are administered to, i.e. the daily dosage for each type of animal.

Example of a calculation of the daily dosage
For example, a farm with 150 fattening pigs with an average weight of 70.2 kg used 2 litres of antibiotic preparation X during the course of one year (40% = 400 mg/ml of which consists of active ingredient a and the remainder of solvent and supplements) and 20 kg of antibiotic preparation Y (25% = 250 mg/g of which consists of active ingredient b). Antibiotic preparation X contains active ingredient a: the specified dose is 10 mg a day per kg animal weight. Antibiotic preparation Y contains active ingredient b: the specified dose is 50 mg a day per kg animal weight.

Antibiotic preparation X can be used to treat (2,000 * 400)/10 = 80,000 kg animal weight. Antibiotic preparation Y can be used to treat (20,000 * 250)/50 = 100,000 kg animal weight. Consequently, the farm has used antibiotics for treatment of a total of 180,000 kg animal weight. The farm has an average of 150 fattening pigs per year, with a total weight of 10,530 kg. 180,000 kg were treated in that year, equivalent to 180,000/10,530 = 17.1 daily dosages. Consequently, an average fattening pig[4] on the farm in that year was administered a prescribed dosage of antibiotics on 17.1 days**.** In this example the farm uses 17.1 daily dosages per animal year of antibiotic preparation X plus Y.

2.4 Statistical analysis

To obtain insight into the amount of antibiotic use on the national level and the trend in this use throughout the years, the sample of farms in FADN and the additional sample for veal calves estimations are used to estimate the usage in the whole population (average use per average animal present on an average farm).

This might raise the question how conclusions can be drawn for the whole population if only a limited number of farms are observed. The answer to this question can be found in the selection of farms that are included in the sample. The farms that are included in the FADN, or in the additional sample for veal calves, should be representative of the whole population. In this way a sample can provide adequate information (Vrolijk et al, 2009).

An important issue is how to ensure that the farms that are included in the sample are representative of the whole population. Therefore a disproportional stratified random sampling strategy is used. A stratified sample implies that the population is divided into a number of groups. Subsequently farms are selected from each of the groups. The variables on which the groups are defined should be relevant variables to make sure that the farms that are included in one group are similar with respect to the important aspects. Using this stratification, and selecting farms from each group, ensures that farms from all groups and consequently with different characteristics are included in the sample. In the FADN sample only farm size is used for the stratification. The additional sample of veal calf farms is also stratified for 'large integration' versus 'small integration or free farms'.

Because of the observed large variation in use of antibiotics between individual farms in the sample relatively large sample sizes are necessary to make reliable estimates for the whole population. In this report the average value for the different sectors in the Netherlands has been calculated with a 95% confidence interval, i.e. on the basis of the sample it can be stated with 95% reliability that the average value for the Netherlands will be between certain lower and upper limits.

[3] For veal calves the calculated daily dosages are based on the highest allowed dosage in stead of the average dosage. This is according daily practice where usually the highest recorded dosage is administered. The use per average veal calf is calculated on the basis of the composition of the veal calf sector in the Netherlands: 70% white veal calves and 30% rosé veal calves.
[4] This refers to a pig on the farm throughout the year: however, there is no such pig. This is a method which can be used to provide for comparisons of farms with different vacancy rates**.** For example, a farm has 2 herds of animals a year, both of which comprise 200 animals that remain on the farm for 5.5 months**.** The farm is vacant during the first and last week of the year, and for 2 weeks between the two herds. The calculations for this farm are based on an average of 183 animals present on the farm. When a farm is vacant for six months and has a herd of 200 animals for six months then the calculations are based on an average of 100 animals on the farm.

In this report the data of this total group of sample farms are used to present the findings about the use of antibiotics and also for further statistical analysis about decrease of increase of antibiotic use over a period of two or more years. Comparing means between two years can be done in two ways, either by only using farms that are in the sample for both years or by comparing the means independently, using all sample farms in both years. The first method usually gives better results if the number of sample farms available in both periods is not much smaller that the number of farms in the separate years. This usually is the case in subsequent years. However, if the years of comparison are further apart, the number of sample farms available in both years will be more limited. Additionally, the direction of the change might even be different from the direction in the total sample. In that case, testing for significant differences can better be done by using the means and standard errors of the separate years instead of using a subsample. If the difference between the two means is larger than twice the square root of sum of both squares of the standard errors then there is a significant difference.

3. Trends in antibiotic usage

3.1 Trends in the total antibiotic use in the Netherlands

Figure 3.1 shows the trends in the total sales of therapeutic antibiotics in the Netherlands**.** The figure was prepared from total sales figures presented by FIDIN (FIDIN, 2009).

Figure 3.1 Veterinary therapeutic antibiotic sales from 1999-2008 (FIDIN, 2009)

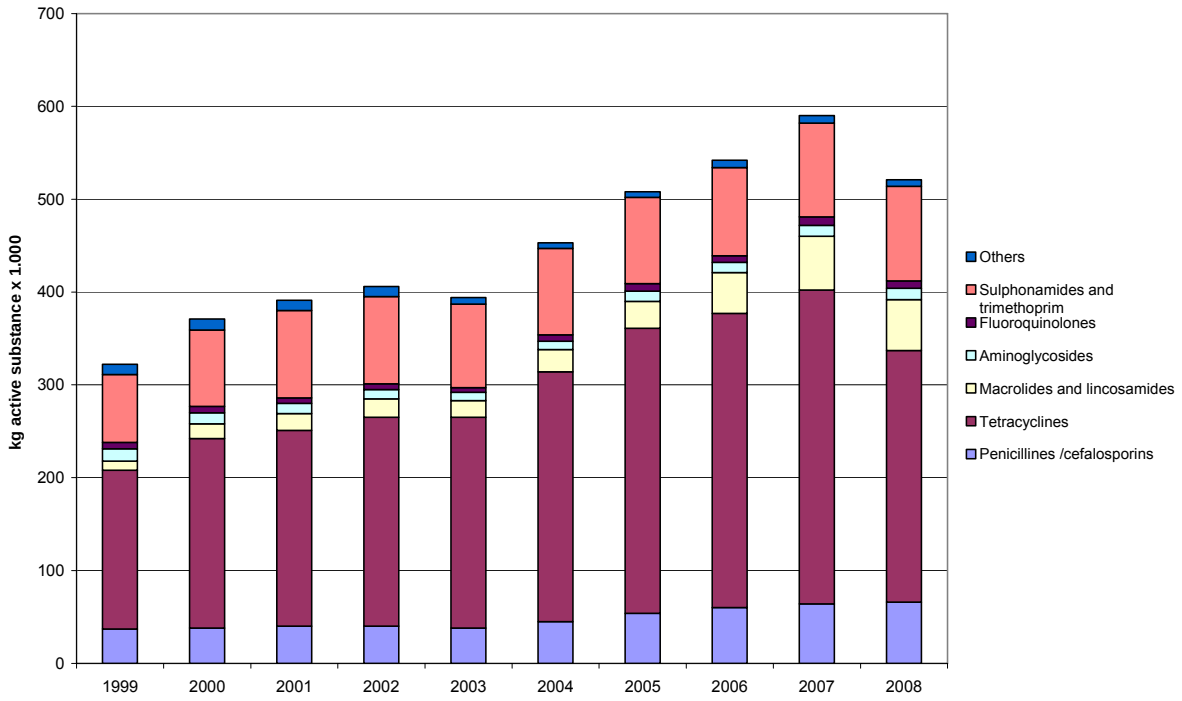


Figure 3.1 reveals that the total amount of antibiotics sold by the pharmaceutical industry in the Netherlands for therapeutic veterinary use has increased in the period 1998-2007. Compared to 2007 in 2008 the amount of antibiotics sold has decreased by 12%. Recently FIDIN presented a further decrease of 2% in the amount sold in 2009 in a press release, while the size of the livestock hardly changed in 2009 compared to 2008.

The use of antimicrobial growth promoters (AMGP) was prohibited at the beginning of 2006. A part of the increase of therapeutic antibiotic use in the years 1998-2006 may be accounted for by a substitution of growth promoters**.**

Over the years, the number of the livestock has also changed. Insight into the trends in therapeutic antibiotic use based on sales figures can be obtained by relating the total data in Figure 3.1 to the trends in the number of animals in the Netherlands. Figure 3.3 shows the trends in the numbers of animals**.**

Figure 3.2 Total sales of antibiotics in the Netherlands, 1998 to 2008.

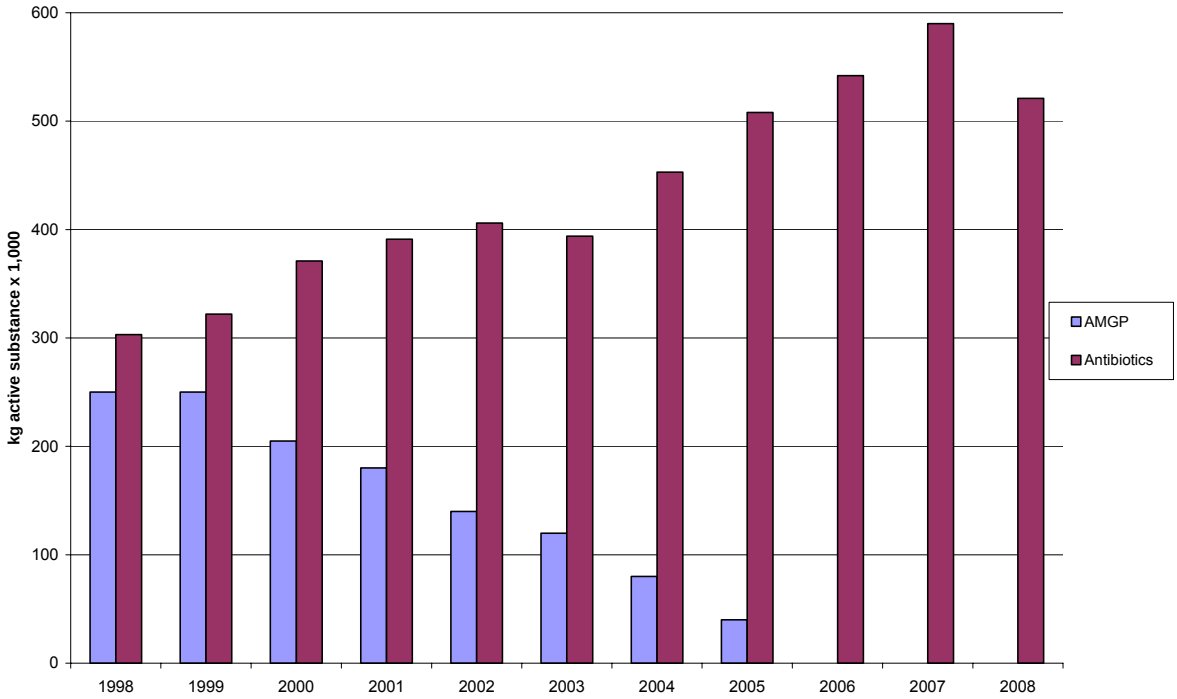
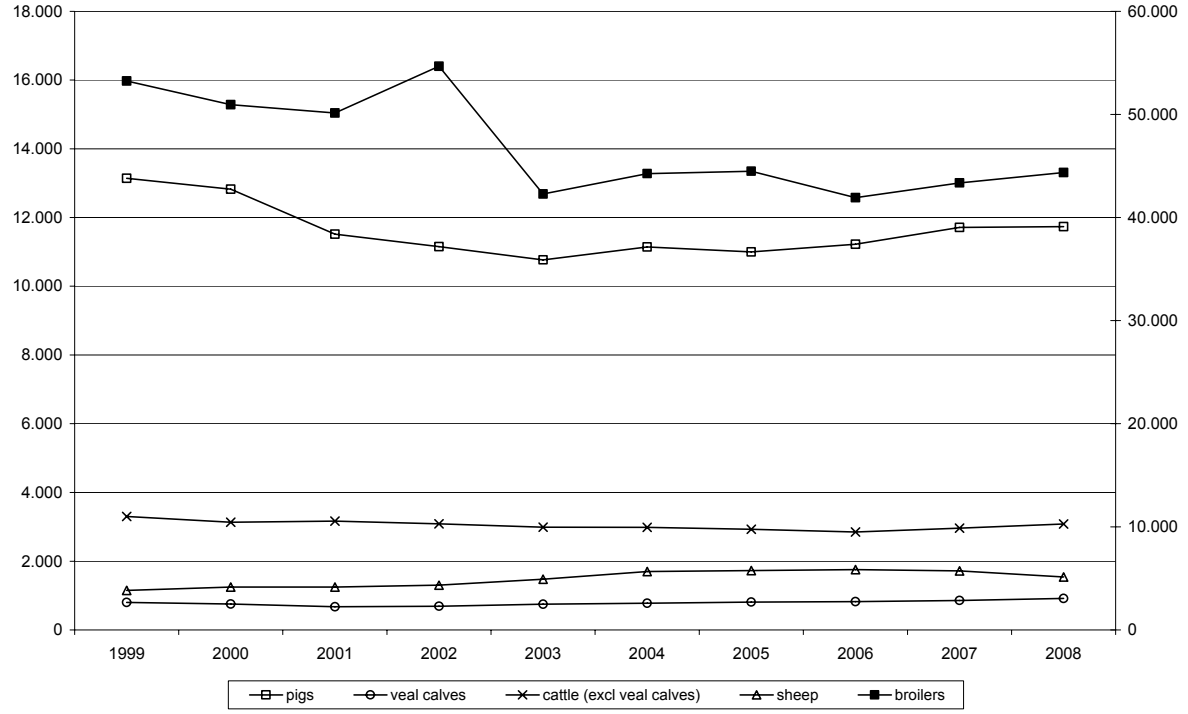


Figure 3.3 Trends in livestock in the Netherlands in numbers of animals, 1999-2008 (x 1,000 animals). The modified scale on the right-hand axis indicates the numbers of broilers (solid squares).



Source: Eurostat 2008.

These yearly numbers of animals are converted into total average live weight in Figure 3.4. Although the number of broilers has fluctuated significantly over the years, the effect on the total weight of the total animal population is minimal (on average, 1.0 kg per broiler). The other types of animal do not exhibit any significant differences**.** The higher total live weights in 1999 and 2000 were due to the relatively larger number of pigs in those years**.**

In conclusion, to obtain insight into the trends in antibiotic usage based on sales figures, the total antibiotic use is divided by the average live weight present (in kg) of the total livestock (see Figure 3.5).

Figure 3.4 Trends in livestock in the Netherlands. Live weight, 1999-2008 (in thousands of kg)

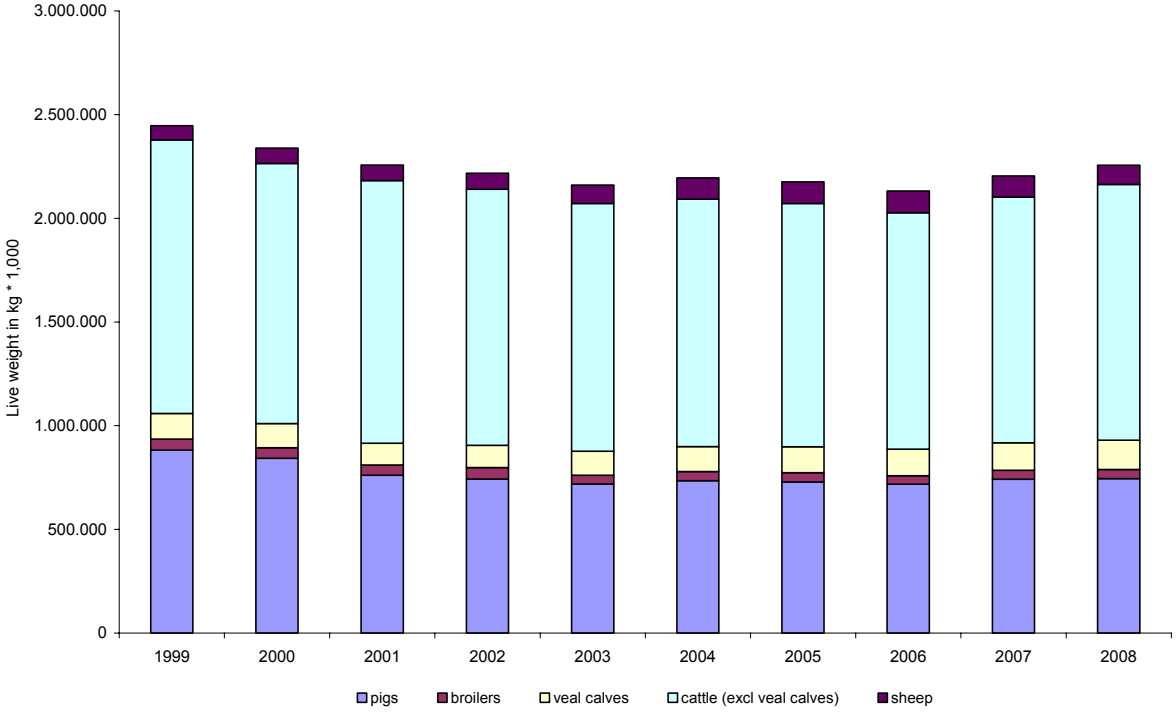


Figure 3.5 Total sales of therapeutic antibiotics 1999-2008, in g per kg live weight

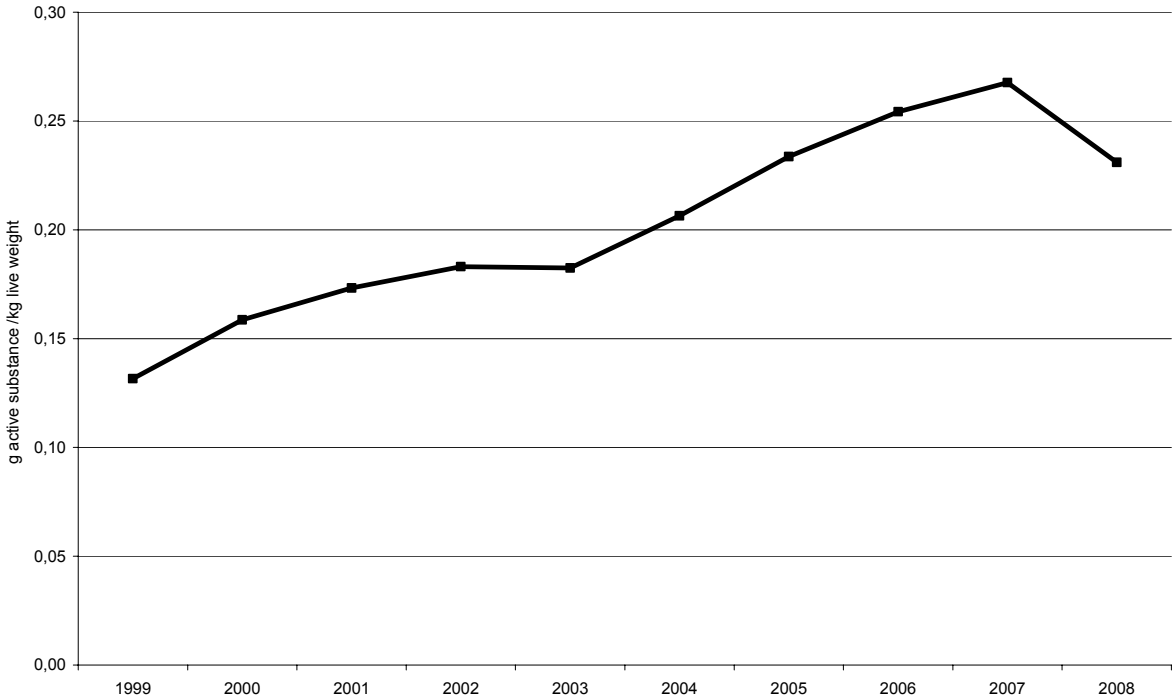


Figure 3.5 reveals that therapeutic antibiotic use expressed in terms of g per kg live weight has doubled in 2007 compared to 1999, but has decreased in 2008 (and 2009) compared to the previous year. FIDIN reports that at least half of the reduction in sales in 2008 compared to 2007 can be explained by stock piling at the veterinarians in the end of the year 2007.

3.2 Trends in exposure to antibiotics in the Netherlands

Figure 3.6 shows the tendencies in exposure to antibiotics in defined daily dosages per average animal present per year (DDDanimal) in the five sectors examined in this study, based on the farms in the samples. The outcome of the calculations is indexed, using 2007 as baseline year. The continuous line represents the calculated average use. The 95% confidence intervals, calculated as from 2005 (indicating that with 95% certainty, the average antibiotic use on a national level, expressed in terms of the number of daily dosages per animal year, will lie within the upper and lower limits) are indicated by the dotted lines shown in Figure 3.6.

Figure 3.6 Tendencies in relative antibiotic usage from 2004-2008 in percentages daily dosages per animal year at the sample farms in 2004 – 2008 (daily dosages per animal year in 2007 = 100%). For veal calves only data from 2007 and 2008 are available.

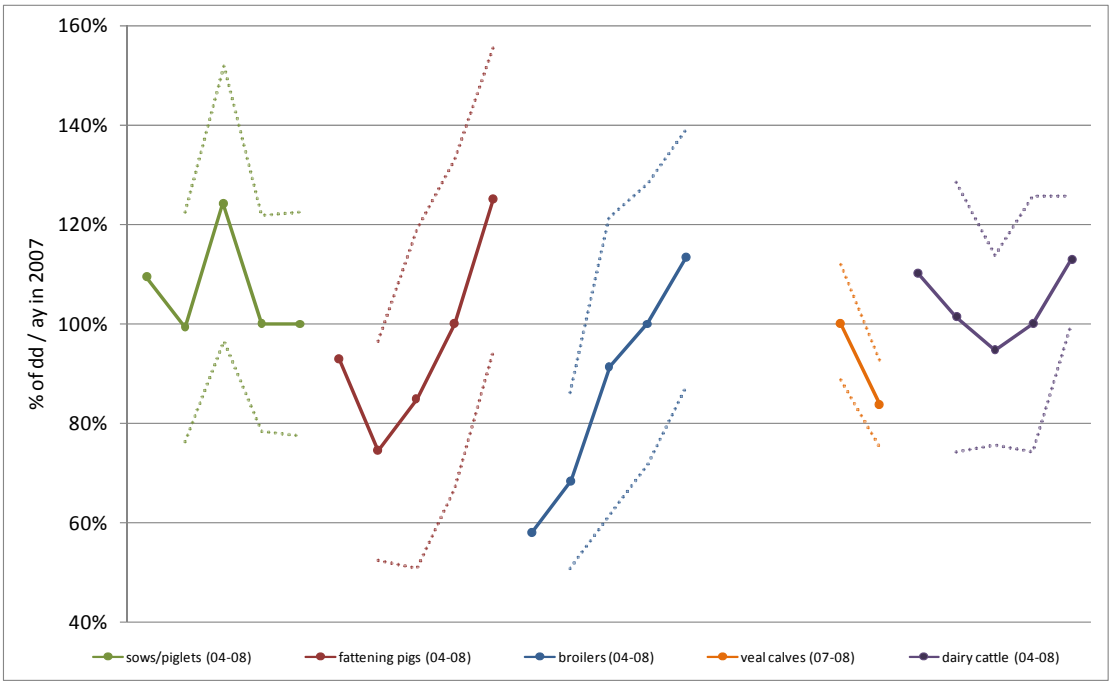


Figure 3.6 shows different tendencies in exposure to antibiotics in the different animal species:
- sow/piglet farms: annual variation with constant usage from 2007 to 2008;
- fattening pig farms: increased usage from the year 2005;
- broiler farms: increased usage from 2004;
- veal calf farms: decreased usage from 2007 to 2008;
- dairy farms: annual variation with increased usage from 2006.

The usage in fattening pigs in 2008 is statistically significantly higher than the use in 2005. Also the usage in broilers in 2008 is statistically significantly higher than the use in 2005.

It is important to note that in spite of the variation presented, the data in figure 3.6 representing the use of antibiotics at the farms in the sample do not permit a conclusion that the use in specific sectors in the Netherlands has increased or decreased in consecutive years. None of the differences between consecutive years were statistically significant. This is primarily due to the observed differences in use between the farms (large variation) in combination with a limited number of farms in the sample. The relatively small confidence interval for veal calves is a favorable consequence of the large sample of veal calf farms. For veal calves only data of the years 2007 and 2008 were available.

3.3 Trends in the total antibiotic use in European countries

Figures for the quantities of sold or prescribed veterinary antibiotics have been published for a number of European countries**.** These countries also present the figures in terms of kg active ingredients**.** The total of these figures is related to the number of livestock in the relevant country. The outcome of the calculations is indexed, using 2007 as base year.

Figure 3.7. Relative amount of sold or prescribed antibiotics per average animal per year in the various countries (g per kg in 2007 = 100%).

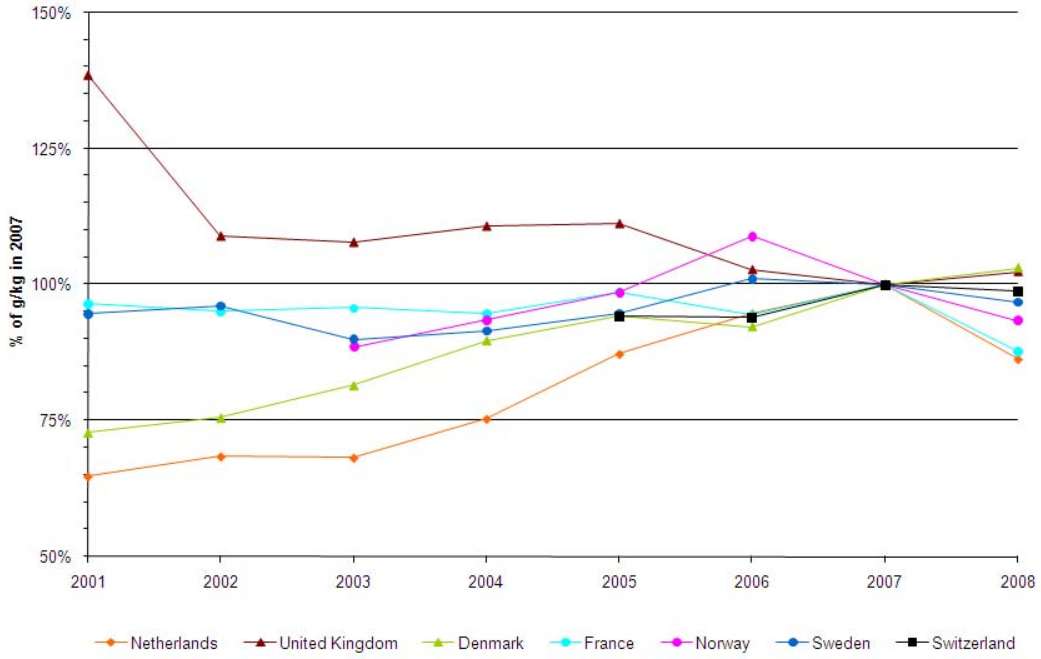


Figure 3.7 shows the trend in sales of antibiotics per average animal per year in the various countries, calculated in % of g per kg live weight in 2007**.** The antibiotic use in 2008 does not differ much from the use in 2007 in most countries, whereas there has been a decrease of more than 5% in Norway, France and the Netherlands.

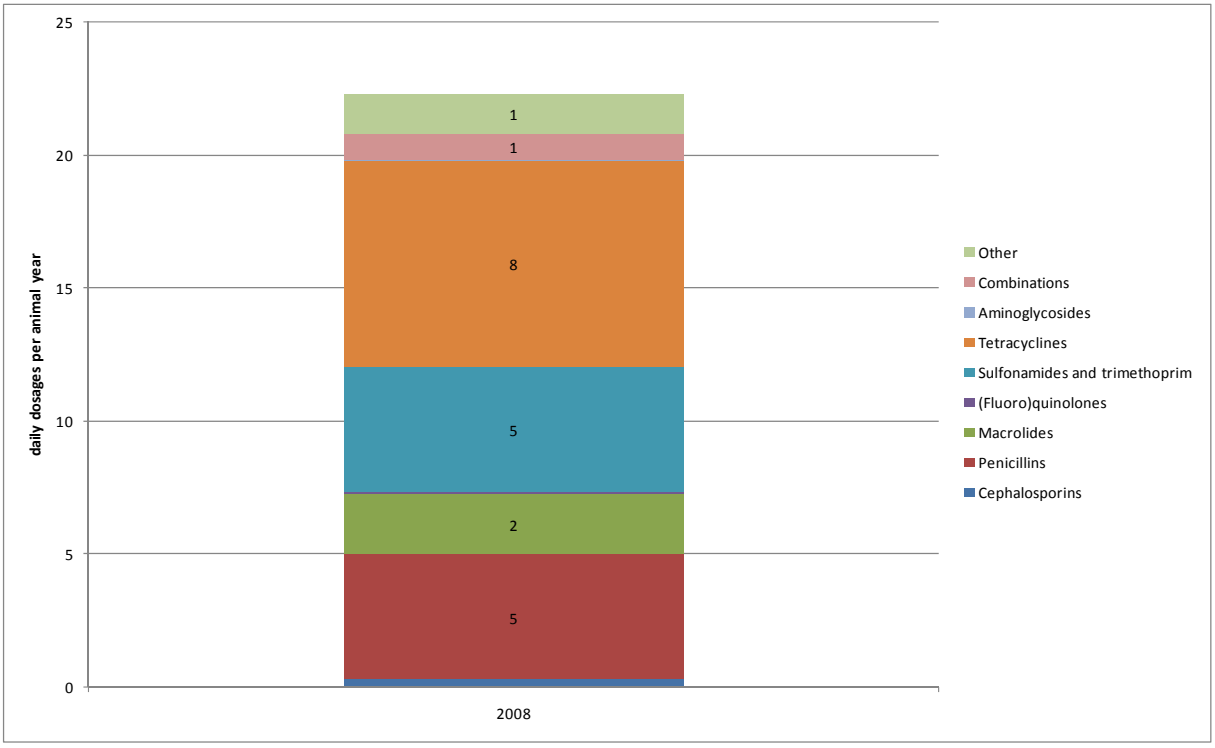
4. Antibiotic usage per animal species in 2008

4.1 Pigs

Sows and piglets
In 2008, the average sow in this survey received approximately 22 daily dosages per year. More than 80% was orally used. The average use in the sow/piglet sector in the Netherlands will be at most 22% higher or lower than the average determined at the sample farms (95% Confidence Interval:17-27 dd/ay). This large confidence interval is mainly caused by the large variation in use that exists between different farms.

Figure 4.1 provides insight in the relative use of the various groups of antibiotics**.**

Figure 4.1 Antibiotic use on sows/piglets in daily dosages per sow per year in 2008



The number in the bar indicates the number of daily dosages per animal year per pharmacotherapeutic group.

Figure 4.1 shows that 35% of the total antibiotic use in sows/piglets in 2008 originates from the administration of tetracyclines, 21% from penicillins and another 21% from sulphonamides and trimethoprim.

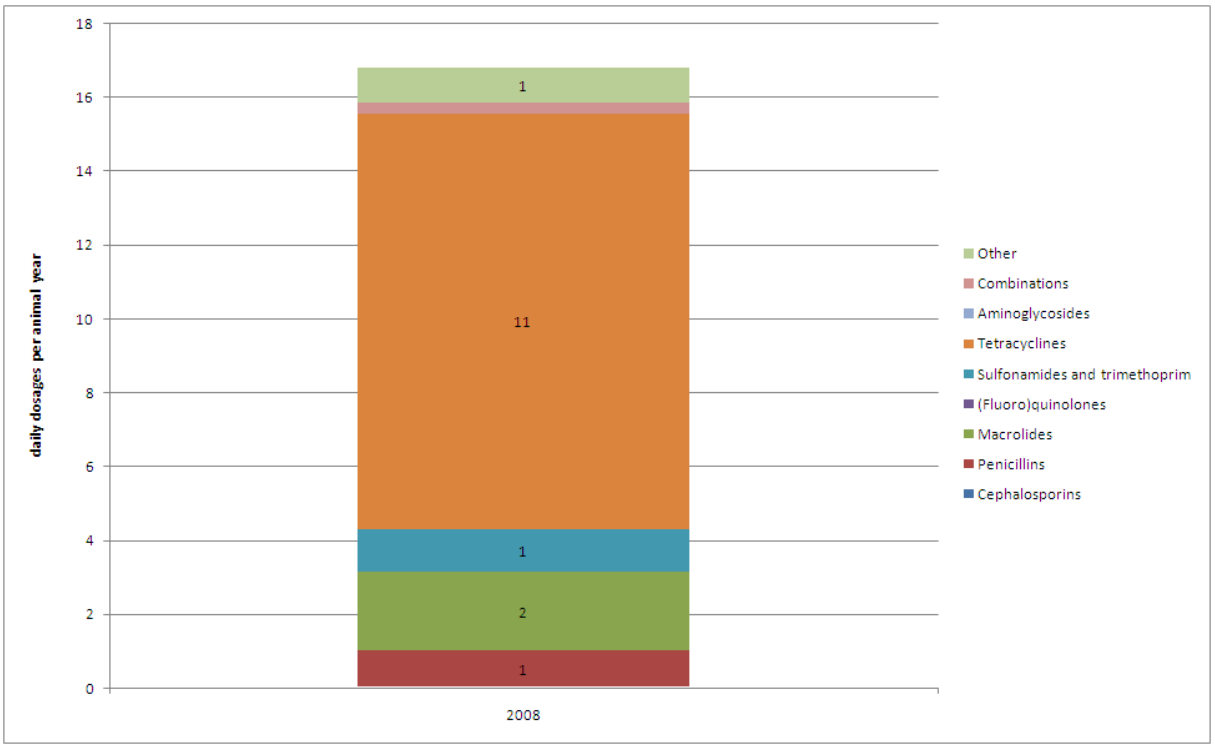
Further analysis reveals that there is a tendency to increasing use of third and fourth generation cephalosporins (0.08 to 0.27 dd/ay) in the period 2005-2008, more specifically of ceftiofur. The increase of ceftiofur is statistically significant.

Discussion
The average number of daily dosages for sows/piglets in the sample in 2008 was 22. However, in practice almost all of the antibiotics are probably used for the treatment of the piglets, not for the sows. In case 100% of the antibiotics are administered to the piglets, with an average weight of 12.5 kg, this would mean that an average piglet is exposed to antibiotics during 30 days in the period from birth to the age of 74 days (at delivering to the fattening farm, at 25 kg).

Fattening pigs
The average fattening pig in the sample received 17 daily dosages per year in 2008, of which nearly 95% oral use. The actual use in the fattening pig sector in the Netherlands will be at most 28% higher or lower than the average determined at the sample farms (95% CI: 12-22 dd/ay).

Figure 4.2 provides insight in the relative use of the various groups of antibiotics in 2008**.**

Figure 4.2 Antibiotic use on fattening pigs in daily dosages per animal year in 2008



The number in the bar indicates the number of daily dosages per animal year per pharmacotherapeutic group.

Figure 4.2 shows that 67% of the total antibiotic use in fattening pigs in 2008 originates from the administration of tetracyclines and 13% from macrolides.

Further analysis reveals that the oral use of tylosin in 2008 is higher than in 2005: 0.58 to 1.88 dd/ay. In the same period we see a tendency to increasing use of tetracyclines (especially doxycycline).

Discussion
The average present fattening pig in the sample received 17 daily dosages per year. Assuming a production period of 117 days, 5 daily dosages are administered to each fattening pig during its production period from 25 kg to slaughter weight. This average fattening pig has also received antibiotics at the breeding farm (30 daily dosages), which brings the total exposure to antibiotics per average fattening pig to approximately 35 days during its whole life from birth to slaughtering at the average age of 191 days.

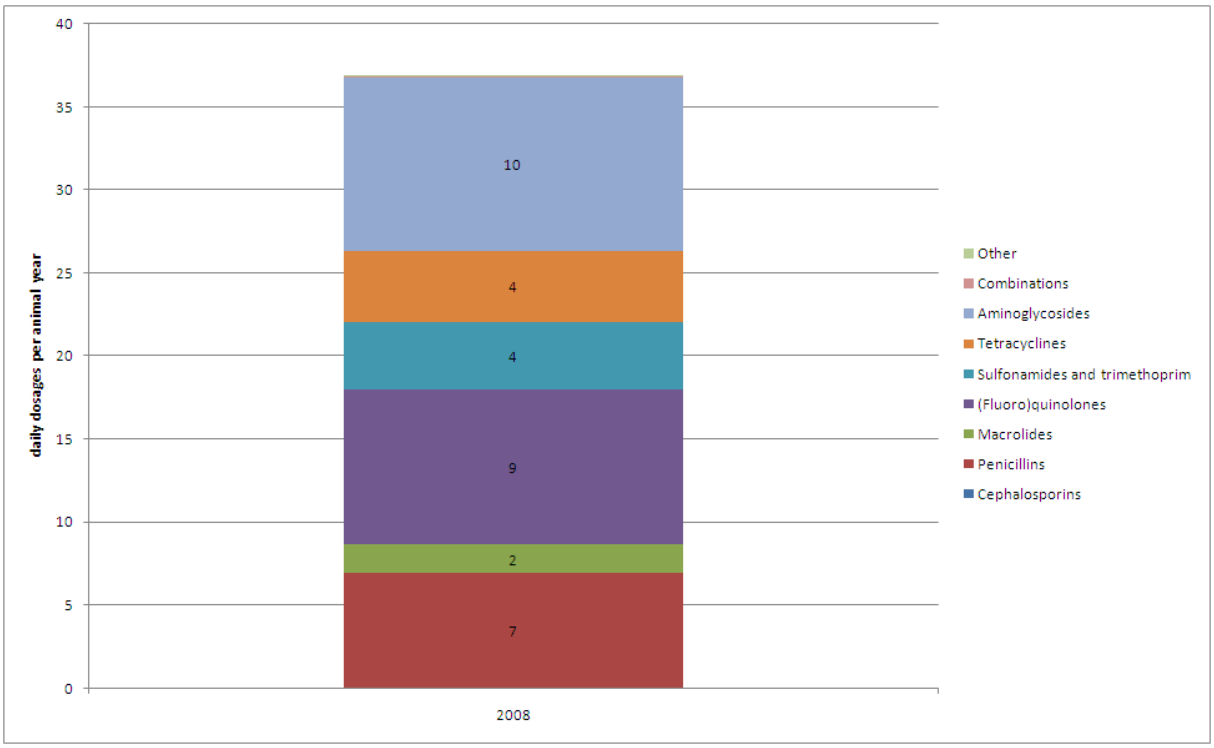
If it is assumed that the average treatment weight of fattening pigs will be thirty percent lower than their average live weight, since younger animals are more likely to receive antibiotics than older animals, the estimation of the total life time actual exposure increases from 35 days to a total of 37 days.

4.2 Broilers

The average boiler chicken in the sample received 37 daily dosages per year in 2008, administered orally, mainly through the drinking water. The actual use in the broiler sector in the Netherlands will be at most 24% higher or lower than the average determined at the sample farms (95% CI: 28-46 dd/ay).

Figure 4.3 provides insight in the relative use of the various groups of antibiotics**.**

Figure 4.3 Antibiotic use on broiler farms in daily dosages per animal year in 2008



The number in the bar indicates the number of daily dosages per animal year per pharmacotherapeutic group.

Figure 4.3 shows that administration of aminoglycosides accounted for 28% of the total antibiotic use on broiler farms in 2008, quinolones for 25% (of which 1.3% fluoroquinolones) and penicillins for 19%.

Further analysis shows a statistically significant increase in the use of aminoglycocides from 2.24 in 2005 to 10.40 dd/ay in 2008. In the same period there are tendencies to increase in the use of penicillins (4.19 to 6.88 dd/ay) and (fluoro)quinolones (7.05 to 9.31 dd/ay).

Discussion

The average present broiler in the sample is administered 37 daily dosages of antibiotics per year. Assuming a number of 7 production periods per year an individual broiler is exposed to antibiotics during 5 days in the period from day one to the slaughter age of 42 days.

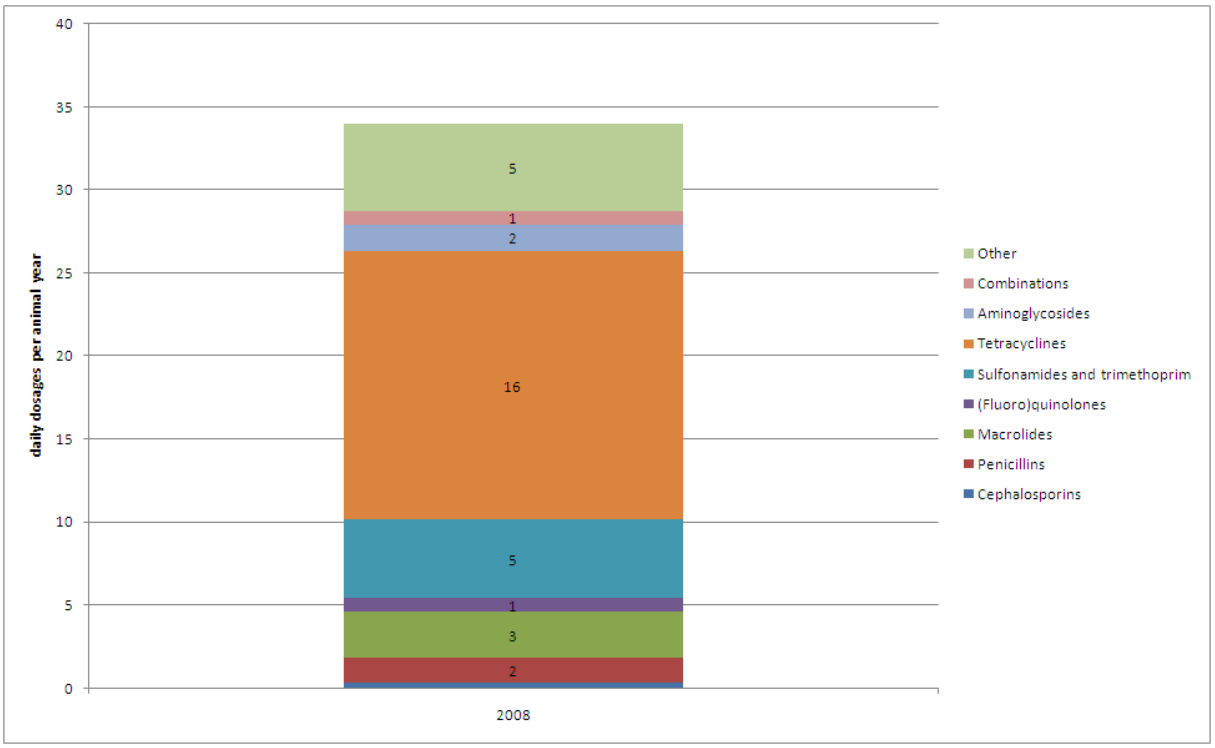
Detailed data reveal that the average treatment weight of broilers equals the average live weight of 1.0 kg. Therefore the calculated exposure of approximately 5 days per broiler can be considered as an adequate estimation of the actual exposure.

4.3 Veal calves

The average veal calf in the sample received 34 daily dosages per animal year in 2008, of which more than 90% was administered by oral route. The actual use in the veal calf sector in the Netherlands will be at most 10% higher or lower than the average determined at the sample farms (95% CI: 31-38 dd/ay).

Figure 4.4 provides insight in the relative use of the various groups of antibiotics**.**

Figure 4.4 Antibiotic use on veal calf farms in daily dosages per animal year in 2008



The number in the bar indicates the number of daily dosages per animal year per pharmacotherapeutic group.

Figure 4.4 shows that 48% of the total antibiotic use on veal calf farms originates from the administration of tetracyclines and 14% from sulphonamides and trimethoprim. In the relatively large group 'others' mainly colistin is important.

Further analysis shows tendencies to decrease in the use of tetracyclines (from 21.1 in 2007 to 16.2 dd/ay in 2008) and third and fourth generation cephalosporins (0.4 to 0.3 dd/ay) and a tendency to increase in the use of fluoroquinolones (0.4 to 0.6 dd/ay).

Discussion

The average veal calf in the sample is administered 34 daily dosages per year. Assuming 1,5 production periods per year (white and rosé) an individual veal calf is exposed to antibiotics during 23 days in the period from birth to the average slaughter age of 222 days.

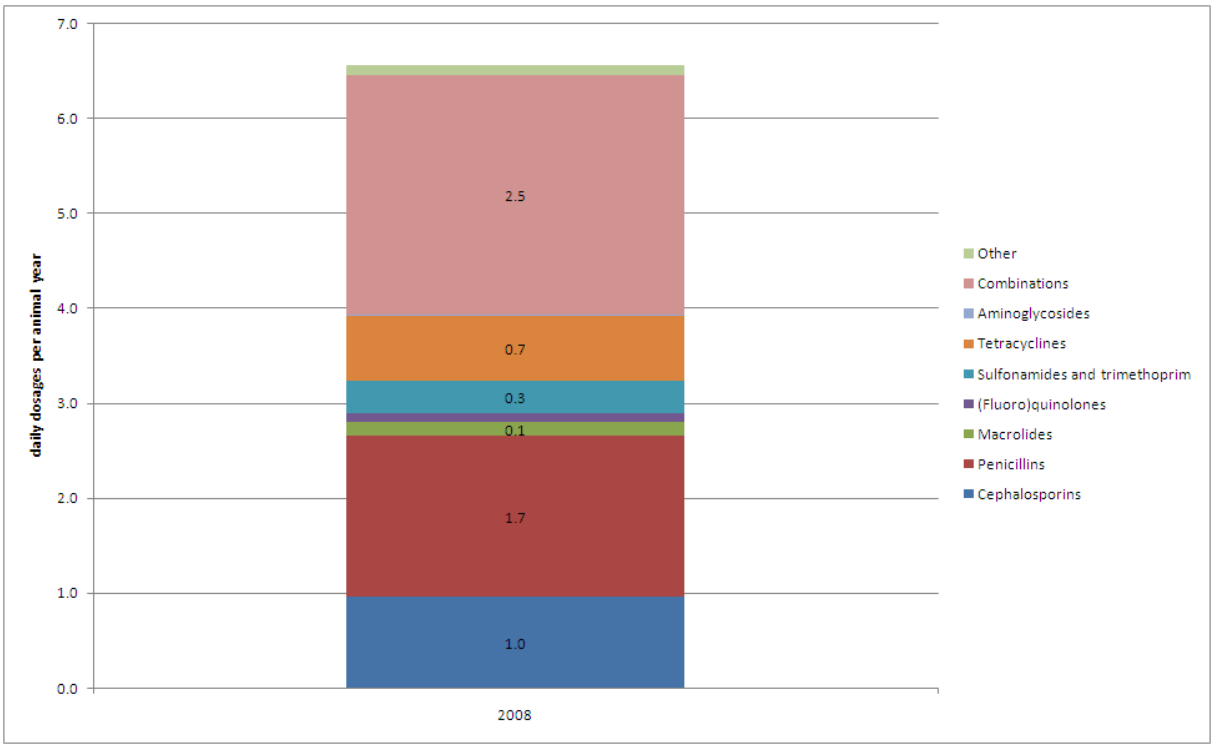
If it is assumed that the average treatment weight of veal calves will be around fifty percent lower than the average live weight, since younger animals are more likely to receive antibiotics than older animals, the estimation of the actual exposure increases from 23 days to a total of 46 days.

4.4 Dairy cows

The average dairy cow in the sample received 6.6 daily dosages per year in 2008. The actual use in the dairy cattle sector in the Netherlands will be at most 13% higher or lower than the average determined at the sample farms (95% CI: 5.8-7.5 dd/ay).

Figure 4.5 provides insight in the relative use of the various groups of antibiotics**.**

Figure 4.5 Antibiotic use on dairy farms in daily dosages per animal year in 2008



The number in the bar indicates the number of daily dosages per animal year per pharmacotherapeutic group.

Figure 4.5 shows that 38% of the total antibiotic use on dairy farms consists of combination therapy (mostly penicillins with aminoglycosides for intramammary therapy) and 26% originates from the administration of penicillins.

Further analysis reveals that there is a tendency to increasing use of third and fourth generation cephalosporins (0.55 to 0.94 dd/ay), more specifically of ceftiofur. The increase of ceftiofur in the period 2005-2008 is statistically significant.

Discussion
The average number of daily dosages per dairy cow per year in the sample was 6.6 in 2008, of which 3.8 for intramammary use and 0.2 orally administered. If it is assumed that the oral use is only applied in young calves, an average calf is exposed to antibiotics during 7 days of the 56 day weaning period. 67% of the intramammary use is used for drying off, which means that on average more than 90% of the dairy cows has received dry cow treatment in all four quarters.

5. Conclusions

The results from the monitoring provide an overview of the total sales of antibiotics in the Netherlands and other European countries. On the basis of monitoring at a sample of farms there is also more detailed information about the (trends in) exposure to various antibiotics at dairy, pig, broiler and veal calf farms.

Trends in total sales of antibiotics in the Netherlands
Therapeutic antibiotic use expressed in terms of grams per kg live weight has doubled in 2007 compared to 1999, but has decreased in 2008. Recent sales figures reveal that in 2009 there has been a further slight decrease. During this same period, the antimicrobial growth promoters have been banned, first partly and as from 2006 entirely.

Tendencies in exposure to antibiotics in the Netherlands
The figures on exposure to antibiotics in the Netherlands expressed in terms of daily dosages per animal per year in the samples reveal the following tendencies for the years 2004 to 2008:
- sow/piglet farms: annual variation with constant usage from 2007 to 2008;
- fattening pig farms: increased usage from the year 2005;
- broiler farms: increased usage from 2004;
- veal calf farms: decreased usage from 2007 to 2008;
- dairy farms: annual variation with increased usage from 2006.

The usage in fattening pigs in 2008 is statistically significantly higher than the use in 2005. Also the usage in broilers in 2008 is statistically significantly higher than the use in 2005.

Trends in the total sales of antibiotics in European countries
The comparison of several European countries for which figures about veterinary antibiotics are available shows that the sales in 2008, expressed in grams per kg live weight, does not differ much from 2007 in most countries, whereas there has been a decrease of more than 5% in Norway, France and the Netherlands.

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Annexes

Additional information regarding the calculation of the daily dosages per animal year can be found in the annexes.
Revised annexes to Part I Usage of antibiotics in animal husbandry in the Netherlands are available on the CVI website at www.maran2008.wur.nl.

II Resistance data

In this chapter susceptibility test results are presented as determined in 2008 for the food-borne pathogens *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* O157, the food-borne commensal organisms *E. coli*, *Enterococcus faecium* and *E. faecalis*, bovine respiratory disease pathogens *Pasteurella multocida* and *Mannheimia haemolytica* and bovine mastitis pathogens *E. coli*, coliform bacteria, *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus uberis* and *S. dysgalactiae*.

6. Food-borne pathogens

Salmonella spp.

Resistance percentages are presented on salmonella's isolated from humans suffering from clinical infections, food-producing animals and food products from animals as potential sources for distribution to humans via the food chain, and animal feeds as potential source for food-producing animals.

Highlights
In 2008 *S*. Typhimurium and *S*. Enteritidis were the most prevalent serovars in humans, third was the monophasic antigenic variant of Typhimurium: S. enterica subspecies enterica 1,4,5,12:i:-. Pigs and cattle were the most important animal sources of *S*. Typhimurium. Layers (eggs) and foreign travel were the most important sources for *S*. Enteritidis. In broilers and poultry meat products *S*. Java was isolated most frequently, but this particular poultry clone of *S*. Java is rarely found in humans.
In 2008 65 ESBL suspected *Salmonella* strains were found. The isolates belonged predominantly to the serovar *S*. Java (69%, N = 45) all of which were isolated from poultry sources. Other ESBL-suspected serovars were Agona, Infantis, Senftenberg, Typhimurium Ft90, Enteritidis PT 4, Virchow, Kottbus, Senftenberg, Cubana, Rissen and Heidelberg, all from poultry; Stanley, Typhimurium Ft 80, FT 510, Montevideo, S. enterica subspecies enterica 1,4,5,12:i:- and Derby from humans, and Dublin from an unknown source. Up to now, poultry is the only reservoir for ESBL-producing salmonella's in animals, which is associated with the transmission of the genetic determinant between Salmonella and ESBL-producing E. coli in poultry.
In 2008 in strains from human infections, a very striking increase was observed in ciprofloxacin resistance (31.3%) compared to 3.7% in 2006/2007. This increase was solely due to DT104, of which 14% were isolated from various animal sources and 86% from human infections. This is a worrisome development since DT104 is associated with increased virulence. It can be expected that human infections with ciprofloxacin non-wild type isolates cause more complications and treatment failure. The source or sources for this increased incidence is unknown, but not likely a Dutch animals source.

In 2008 a total of 3834 *Salmonella* isolates were sent to RIVM Bilthoven for sero-, and phagetyping, of which 2709 were tested for antimicrobial susceptibility (Table 6.1 and 6.2). Human isolates (N= 1502) 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=917) and cattle, including calves (N=85) were sent to the RIVM by the Animal Health Service from a diversity of surveillance programs and clinical Salmonella infections. Those from chickens (broilers, including poultry products, N=454; layers, reproduction animals and eggs, N=58) concerned mainly nonclinical *Salmonella* infections derived from a diversity of monitoring programs on the farm, slaughterhouses and at retail. A large proportion of isolates from pigs in 2008 concerned those from the Dutch component of the EU-baseline study. Isolates from a diversity of other sources have been analysed as well (animal feed and human food products; other animals from animal husbandry and pets, samples from the environment, etc.).
In 2008 similar to 2006/2007, *S.* Typhimurium and *S.* Enteritidis were the most prevalent serovars isolated from humans in the Netherlands (Table 6.1). Third was again the antigenic monophasic variant of *S.* Typhimurium: *S. enterica subspecies enterica* 1,4,5,12:i:-.
In pigs *S.* Typhimurium was by far the most prevalent serovar, *S.* Derby and *S. enterica subspecies enterica* 1,4,5,12:i:- were second and third. In cattle *S.* Dublin and, on second place, *S.* Typhimurium predominated. In poultry a difference existed in prevalence of serovars between broilers and layers. In broilers *S.* Paratyphi B var. Java (*S.* Java) predominated and in layers *S.* Enteritidis. *S.* Senftenberg showed a striking decrease in prevalence compared to

previous years while S. Typhimurium surprisingly doubled in incidence. However note that the involved numbers of isolates in layers are small.
From 2006 onwards travel contributed from 0% to 33% of the cases of human salmonellosis depending on the sero/phagetype. It contributed substantially more to the incidence of *S.* Enteritidis than *S.* Typhimurium. Travel contributed to 33% of the *S.* Kentucky cases in humans, a serovar associated with trips to Northern African countries, and to 31% of the typhoidal Salmonella cases. However, the contribution of travel as depicted in Table 6.1 is only indicative of the true contribution, because travel is strongly underreported, by about a factor two.

Table 6.1. Most prevalent *Salmonella* sero-, and phagetypes isolated in 2006/2007 and 2008 from humans, pigs, poultry, broilers and layers[5] and the % travel related human infections from 2006 - 2008.

	Travel	Humans		Pigs		Cattle		Poultry		Broilers		Layers	
	>2006	06/07	2008	06/07	2008	06/07	2008	06/07	2008	06/07	2008	06/07	2008
N total	7%	2936	1648	760	917	318	85	1101	672	844	454	177	58
N tested		2476	1502	258	338	250	74	644	427	456	301	129	37
		%	%	%	%	%	%	%	%	%	%	%	%
Typhimurium	3%	32,3	35,2	39,5	13,6	26,1	23,5	4,5	3,9	4,1	2,2	6,2	12,1
Ft506 (DT104)	2%	4,4	11,7	7,8	2,6	5,0	3,5	1,5	0,9	1,7	0,2	1,7	5,2
Ft507	1%	8,0	10,0	7,5	3,1	8,5	4,7	0,9	1,2	0,9	1,1		0,0
Ft561 (DT7)	0%	6,9	0,1	1,1		2,8							0,0
Ft651 (DT15a)	2%	0,1	1,3		0,3		1,2		0,4				3,4
Enteritidis	14%	37,3	34,3	4,1	1,2	2,8		7,0	9,4	3,7	5,7	21,5	39,7
Pt4	7%	15,3	7,5	0,8		0,9		2,8	2,8	1,5	1,1	7,3	19,0
Pt8	11%	2,7	8,7	0,7	0,5	0,6		0,4	2,4	0,2	1,8	0,6	3,4
Pt21	19%	4,7	5,7	0,3		0,3		0,8	1,2	0,1	0,9	4,5	3,4
Pt1	21%	3,1	3,0	0,4	0,1			0,7	0,1	0,7		0,6	1,7
Pt6	17%	4,2	1,9	0,1	0,1			0,1	0,3		0,4	0,6	
Pt14b	19%	0,7	1,8	0,1				0,2		0,1		0,6	
Agona	29%	0,5	0,2	1,4	1,4			1,4	1,2	1,4	1,3	0,6	
Anatum	23%	0,2	0,4	0,8	3,2		1,2	1,3	0,4	1,3	0,7		
Blockley	22%	0,3	0,2		0,1			0,1	0,1	0,1	0,2		
Brandenburg	2%	0,6	0,4	4,2	6,1		1,2		0,1				
Bredeney	7%	0,5		0,7	1,4			0,5	0,1	0,7			
Corvallis	29%	0,5	0,8					0,3	0,1	0,4	0,2		
Derby	7%	0,5	0,2	10,1	18,8		1,2	0,3	0,1	0,4			1,7
Dublin	3%	0,5	0,4	0,1	0,4	56,3	62,4						
Gallinarum	--							2,2	1,2			13,0	10,3
Hadar	25%	0,8	0,3	0,4	0,4			2,5	1,9	3,1	2,6		
Heidelberg	9%	0,5	0,5	0,1	1,1			2,3	1,0	1,3	1,1	5,1	
Infantis	14%	1,0	0,8	4,3	3,6	0,9		13,0	7,7	14,9	7,0	4,5	
Paratyphi B var Java	7%	0,4	0,6	0,3	0,2		2,4	35,4	48,1	42,7	57,3	1,7	1,7
Kentucky	33%	1,0	1,2	0,1				0,5	0,3	0,2	0,2	1,7	
Livingstone	6%	0,2	0,1	2,4	6,3			1,5	1,3	1,8	0,7	1,1	6,9
London	4%	0,2	0,4	3,0	12,2	0,3		0,5		0,7			
Mbandaka	17%	0,4	0,1	0,7	0,7	0,3		2,1	4,3	2,3	6,2	1,7	1,7
Montevideo	24%	0,4	0,2	0,9	0,2	1,6	3,5	0,2		0,2			
Newport	23%	1,3	0,7	0,4	0,2				1,8		1,1		
Rissen	28%	0,1		1,1	0,5			0,1	0,4	0,1	0,2		1,7
Saintpaul	28%	0,5	0,8	0,1	0,1	0,3		2,1	0,4	2,4	0,7	0,6	
Senftenberg	26%	0,5	0,4	0,8	0,2			5,4	0,6	1,5	0,4	24,9	1,7
Tennessee	8%	0,0	0,1	0,1					1,0		0,7		6,9
Thompson	9%	0,2	0,4	0,5				1,0	0,7	0,4	0,7	4,5	
Virchow	34%	1,6	1,3	0,4	0,1			6,6	1,2	7,0	1,3	4,5	1,7
Weltevreden	25%	0,4	0,3										
Goldcoast	2%	0,4	0,1	1,1	7,1	1,6		0,1		0,1			
Fluntern	--							0,5				2,8	
Panama	6%	0,4	2,1	1,2	0,9	0,6			0,1		0,2		
SI 1,4,5,12:i:-	2%	5,1	5,8	10,3	2,7	5,7		1,0	2,1	1,1	1,5		
(Para)Typhi (A B C)	31%	1,5	1,3										
Other		9,7	10,4	10,9	17,1	3,5	4,7	7,8	10,0	8,2	7,7	5,6	13,8

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**.**

[5] Source: Report on trends and sources of zoonotic agents in the EU, 2008, The Netherlands

Table 6.2. MIC distribution (in %) and resistance percentages (R%) for all salmonella's (N =2709) tested for antibiotic susceptibility in 2008.

Salmonella N = 2709	MIC (%) distribution mg/L																		
	0.015	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	R%
Ampicillin						2.4	38.9	29.3	2.2	0.2		0.1	26.8						27.2
Cefotaxime			39.5	48.4	8.4	1.2	0.1	0.1	0.1	2.1									2.4
Ceftazidime					56.2	38.1	3.3	0.2	0.7	0.3	0.5	0.8							2.2
Gentamicin					18.8	61.1	16.9	1.8	0.2		0.6	0.4	0.3						1.5
Kanamycin									91.8	5.1	1.0		0.1		2.0				3.2
Streptomycin								6.3	16.8	21.0	21.6	8.9	6.0	7.6	11.8				25.5
Tetracycline							7.6	53.2	10.8	1.3	0.1	5.5	4.4	17.1					27.1
Sulphamethoxazole										45.8	19.5	2.1	0.1	0.1		0.2	0.3	31.9	32.4
Trimethoprim						80.2	1.7	0.2	0.1				17.8						17.9
Ciprofloxacin	11.4	67.3	3.7	1.1	10.9	3.4	1.3	0.5		0.1	0.3								17.6
Nalidixic acid									72.1	10.4	0.8	0.3	0.1	16.4					16.8
Chloramphenicol								0.6	42.4	44.5	3.2	2.1	6.1	1.1					9.3
Florfenicol								0.1	5.4	71.9	10.9	0.3	0.2	11.2					11.7
Colistin										100									0

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

Table 6.2 presents MIC-distributions and resistance percentages of all salmonella's tested for susceptibility in 2008. Highest levels of resistance were observed for sulphamethoxazole, ampicilline, tetracycline, streptomycin and to a lesser extend trimethoprim, ciprofloxacin, nalidixic acid and florfenicol.

In 2008 65 cefotaxime reduced susceptible (MIC > 0.5 mg/L), ESBL suspected strains were found, of which the majority was resistant based on EUCAST clinical breakpoints. The isolates belonged predominantly to the serovar *S.* Java (69%, N = 45) of which all were isolated from poultry sources. Other ESBL-suspected serovars were Agona, Infantis, Senftenberg, Typhimurium Ft90, Enteritidis Pt4, Virchow, Kottbus, Senftenberg, Cubana, Rissen and Heidelberg, all from poultry; Stanley, Typhimurium Ft80, Ft510, Montevideo, *S. enterica subspecies enterica* 1,4,5,12:i:- and Derby from humans, and Dublin from cattle. Twenty eight of these isolates (43%) were resistant against nalidixic acid and also showed increased MICs for ciprofloxacin (MIC 0.25 – 2 mg/L). Four isolates (3 Java, 1 Rissen, 1Derby) showed a phenotype typical for the presence of plasmid mediated quinolone resistance (MIC ciprofloxacin 0.5 – 2 mg/L and nalidixic acid 8 – 32 mg/L). The ESBL and qnr genes were confirmed in one *S.* Java by PCR and sequenced as $bla_{\text{CTX-M-9}}$, and *qnrA* on a large IncHI2 plasmid.

Resistance against cefotaxime in isolates from poultry is increasing at an alarming rate. This is associated with transfer of ESBLs between *E. coli* and *Salmonella* in the GI-tract of Dutch poultry.

Using the epidemiological cut off value of 0.06 mg/L, 478 isolates (18%) were detected that demonstrated a non-wild type phenotype for ciprofloxacin. Of these 62 (2.3%) showed MICs larger that the clinical breakpoint (1 mg/L). The serovars of these ciprofloxacin resistant isolates were predominantly travel related *S.* Kentucky (21%) and *S.* Java from poultry (48%). Since 2002 annually high-level ciprofloxacin resistant *S.* Kentucky's were isolated from human patients. These strains are related to travel to North African countries and are genetically closely related.

Table 6.3. Resistance (%) of the fourteen most prevalent *Salmonella* serovars isolated in the Netherlands in 2008.

	Typhimurium (601)	Enteritidis (539)	Java (215)	subsp. enterica 1,4,[5],12:i:- (122)	Derby (81)	Infantis (73)	Dublin (68)	Senftenberg (47)	Livingstone (44)	Panama (44)	Mbandaka (43)	London (41)	Virchow (35)	Brandenburg (34)
Ampicillin	61.4	3.0	62.3	82.8	11.1	9.6	2.9	4.3	4.5	4.5	2.3	7.3	22.9	0
Cefotaxime	0.5	0.2	20.9	0.8	1.2	2.7	1.5	2.1	0	0	0	0	2.9	0
Ceftazidime	0.2	0	20.0	1.6	1.2	2.7	1.5	0	0	0	0	0	2.9	0
Gentamicin	0.3	0.2	2.8	0.8	1.2	4.1	1.5	0	0	0	0	0	5.7	0
Kanamycin	2.0	0.7	7.9	4.1	1.2	6.8	1.5	0	2.3	2.3	2.3	2.4	11.4	0
Streptomycin	62.2	1.1	36.7	82.0	8.6	9.6	7.4	2.1	9.1	0	0	9.8	8.6	17.6
Tetracycline	70.2	0.7	27.0	81.1	16.0	6.8	2.9	4.3	4.5	6.8	2.3	4.9	25.7	20.6
Sulphamethoxazole	72.5	1.3	63.7	90.2	28.4	13.7	5.9	2.1	18.2	4.5	9.3	34.1	31.4	20.6
Trimethoprim	20.8	0.6	92.6	18.9	25.9	12.3	0	0	18.2	4.5	4.7	34.1	31.4	11.8
Ciprofloxacin	25.3	14.8	57.7	1.6	3.7	4.1	7.4	2.1	2.3	0	0	0	68.6	2.9
Nalidixic acid	25.0	14.8	53.0	0.8	3.7	2.7	5.9	0	2.3	0	0	0	68.6	2.9
Chloramphenicol	43.3	0.4	4.2	5.7	3.7	1.4	4.4	0	2.3	0	2.3	2.4	2.9	0
Florfenicol	38.3	0.2	2.8	1.6	1.2	0	1.5	0	0	0	0	0	0	0
Colistin	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0

In Table 6.3 resistance percentages are presented for the twelve most prevalent serovars isolated in The Netherlands in 2008. As in 2007, the highest resistance levels are observed in *S.* Typhimurium, *S.* Java, the monophasic *S. enterica subspecies enterica* 1,4,[5],12:i:-, and *S.* Virchow.

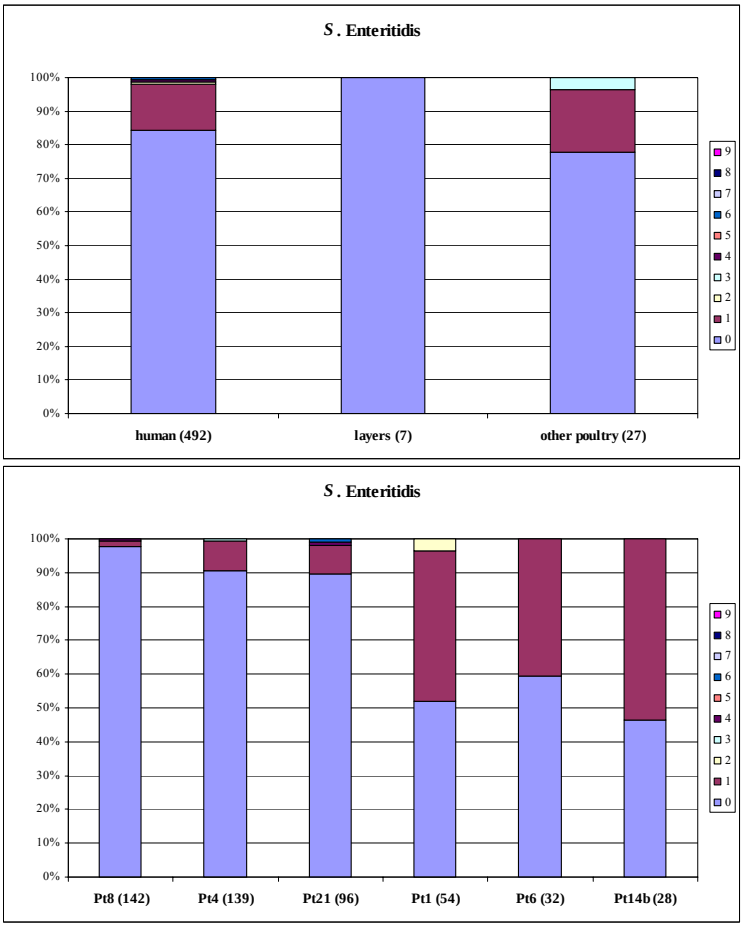
S. Enteritidis

Table 6.4. Resistance (%) of *S.* Enteritidis isolated from humans and poultry and phage types 8, 4, 21, 1, 6, and 14b isolated from different sources in 2008.

	***S*. Enteritidis**			**Most prevalent phage types**					
	Human (492)	**Layers (7)**	**Other poultry (25)**	**Pt8 (142)**	**Pt4 (139)**	**Pt21 (94)**	**Pt1 (54)**	**Pt6 (32)**	**Pt14b (28)**
Ampicillin	2.6	0	4.0	0.7	1.4	2.1	5.6	0	0
Cefotaxime	0	0	4.0	0	0.7	0	0	0	0
Ceftazidime	0	0	0	0	0	0	0	0	0
Gentamicin	0	0	0	0	0	0	0	0	0
Kanamycin	0.8	0	0	0.7	0	2.1	1.9	0	0
Streptomycin	1.2	0	0	0.7	0	2.1	0	0	0
Tetracycline	0.8	0	0	0	0	1.0	0	0	0
Sulphamethoxazole	1.2	0	0	0.7	0	2.1	0	0	0
Trimethoprim	0.4	0	0	0	0	0	0	0	0
Ciprofloxacin	14.0	0	24.0	1.4	8.6	8.3	44.4	40.6	53.6
Nalidixic acid	14.0	0	24.0	1.4	8.6	8.3	44.4	40.6	53.6
Florfenicol	0.2	0	0	0	0	1.0	0	0	0
Chloramphenicol	0.4	0	0	0	0	1.0	0	0	0
Colistin	0	0	0	0	0	0	0	0	0

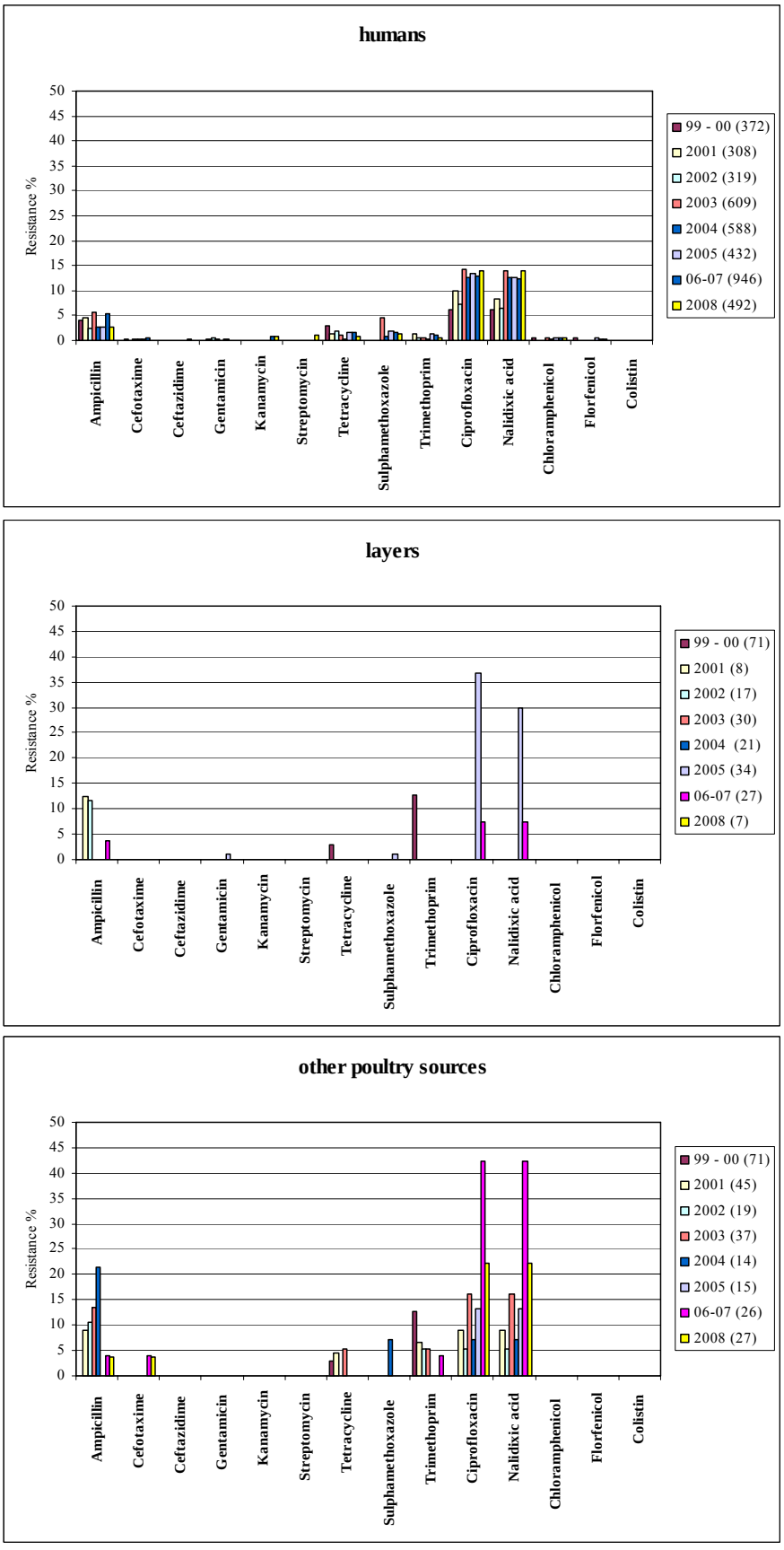
In Table 6.4 resistance percentages for *S.* Enteritidis and it most prevalent phage types are presented.
In the Netherlands, human infections caused by *S.* Enteritidis are predominantly related to the consumption of raw shell eggs. In Dutch broilers and broiler products the prevalence of *S.* Enteritidis is substantially lower than *S.* Java (Tables 6.1 and 6.7). The difference in numbers isolated from Dutch poultry and the resistance profile of strains from human infections and Dutch poultry indicates that other sources of infection exist, amongst them travel (Table 6.1) and imported eggs. In 2008, 69 ciprofloxacin non wild type susceptible strains were isolated from human infections, predominantly Pt1 (35%), to a lesser extend Pt14b (22%) and Pt6 (17%). Pt4, which was in second place (16%) in 2006/2007 occurred only three times (4%) in 2008.
Ciprofloxacin and nalidixic acid resistance were observed most frequently in the human isolates, which was mainly related to phage types Pt1, Pt14b and Pt6. In these phage types multidrug resistance was also most frequently observed. Although 'other poultry', which included broilers, may have contributed to the ciprofloxacin non-wild type/nalidixic acid resistant isolates found in humans, the vast majority of the human infection with ciprofloxacin non-wild type/nalidixic acid resistant isolates have a non-domestic source, being either travel related or related to imported contaminated egg-, or poultry products.

Figure 6.1. Percentages of *S*. Enteritidis strains fully susceptible, resistant to one to nine different antibiotic classes in human and animal sources in the Netherlands in 2008, presented by source (top figure) or phage type (lower figure).



In isolates from human infections the resistance levels remained stable, while in isolates from poultry annual variation can be observed, mainly in resistance against the quinolones (Figure 6.2). However, because of the small numbers of isolates each year, any conclusions on trends observed are unreliable.

Figure 6.2. Trends in resistance (%) of *S.* Enteritidis isolated from humans, layers and other poultry sources from 1999 – 2008.



***S.* Typhimurium**

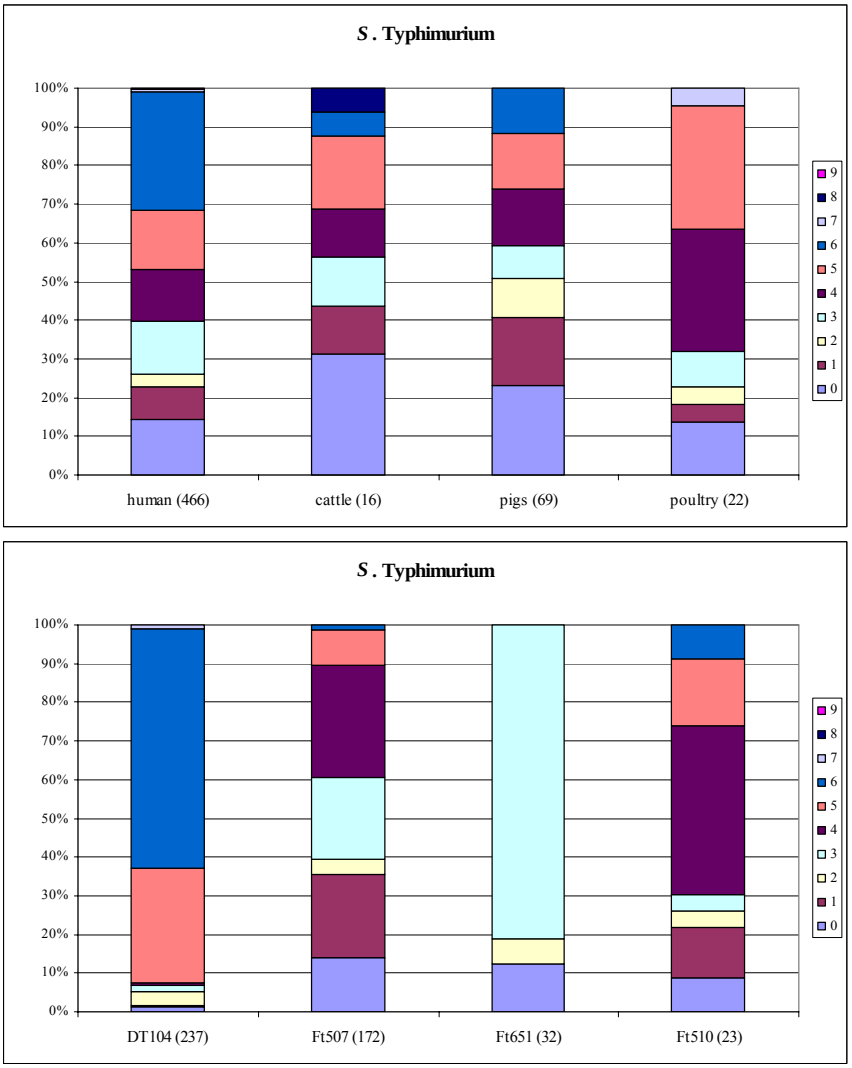
Table 6.5. Resistance percentages of *S.* Typhimurium isolated from different sources and the most prevalent phage types in 2008.

	***S*. Typhimurium**				**Phage types**			
	human (466)	**cattle (16)**	**pigs (69)**	**poultry (22)**	**DT104 (237)**	**Ft507 (172)**	**Ft651 (32)**	**Ft510 (23)**
Ampicillin	63.7	50.0	47.8	63.6	96.6	43.6	0	69.6
Cefotaxime	0.4	0	0	4.5	0	0	0	4.3
Ceftazidime	0	0	0	4.5	0	0	0	0
Gentamicin	0.2	6.3	0	0	0	0	0	0
Kanamycin	1.9	6.3	2.9	0	0.4	2.9	0	4.3
Streptomycin	65.5	50.0	39.1	77.3	94.5	45.3	81.3	52.2
Tetracycline	70.8	68.8	62.3	72.7	93.7	56.4	81.3	78.3
Sulphamethoxazole	76.0	56.3	55.1	77.3	98.3	61.6	84.4	78.3
Trimethoprim	19.3	12.5	30.4	27.3	5.9	41.9	3.1	26.1
Ciprofloxacin	31.3	0	1.4	13.6	59.1	1.7	3.1	4.3
Nalidixic acid	31.3	0	1.4	4.5	59.1	1.2	3.1	4.3
Florfenicol	42.3	25.0	23.2	27.3	92.4	0	3.1	17.4
Chloramphenicol	47.9	31.3	24.6	31.8	92.8	9.3	3.1	30.4
Colistin	0.2	0	0	0	0.4	0	0	0

In 2006/2007 and 2008 the most predominant phage types of *S.* Typhimurium in the collection of strains received from RIVM Bilthoven were: Ft506 (≈ DT104), Ft507, Ft651 (≈ DT15a) and Ft560, all involved in food-borne explosions (Table 6.5).

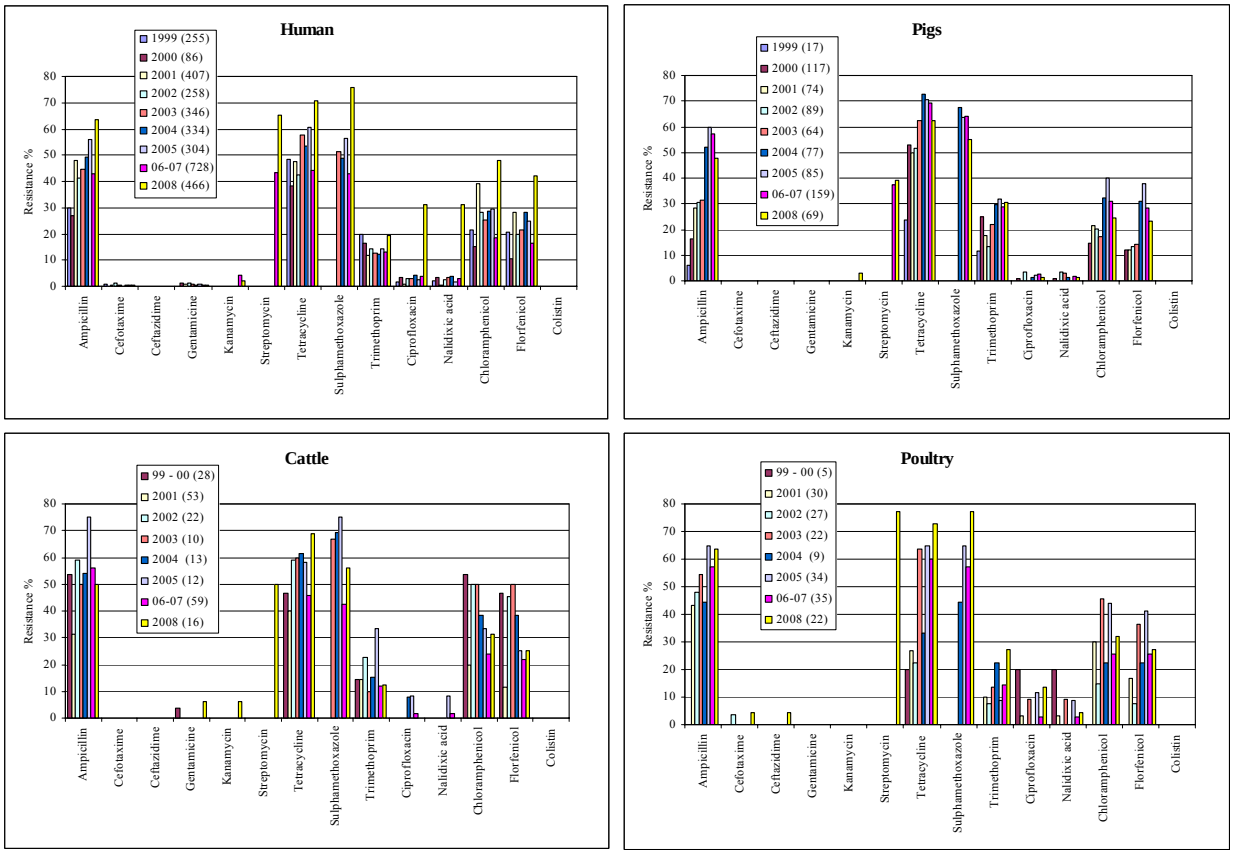
The occurrence of resistance is much more common in *S.* Typhimurium than in *S.* Enteritidis. A typical resistance pattern for *S.* Typhimurium is irrespective of the phage type ASTSuCipNalFC (Table 6.5). Ft 651 (DT15a) showed a different R-type: STSu without resistance against ampicillin, which is a rare phenomenon in Typhimurium. Resistance against 3rd generation cephalosporins was again predominantly observed in isolates from poultry.
In 2008 in strains from human infections, a very striking increase was observed in ciprofloxacin non-wild type isolates (31.3%) compared to 3.7% in 2006/2007. This increase was solely due to DT104, of which 14% were isolated from various animal sources and 86% from human infections. This is a worrisome development since DT104 is associated with increased virulence. It can be expected that human infections with ciprofloxacin non-wild type isolates cause more complications and treatment failure. The source or sources for this increased incidence is unknown, but not likely a Dutch animals source. The involved strains concerned a foodborn outbreak at the end of 2008 of which the MLVA pattern points to quinolone resistant DT104 strains involved in outbreaks abroad.
Multiple resistances occur substantially more frequent in *S.* Typhimurium than in *S.* Enteritidis (Figures 6.1 and 6.3). Of the *S.* Typhimurium strains, 60% (humans), 44% (cattle), 41% (pigs) and 68% (poultry) and were resistant to three or more antibiotic classes (Figure 6.3).

Figure 6.3. Percentages of *S.* Typhimurium strains fully susceptible, resistant to one to nine different antibiotic classes in human and animal sources in the Netherlands in 2008, presented by source (top figure) or phage type (lower figure).



Resistance in *S.* Typhimurium shows a clear tendency to increase in strains from humans (Figure 6.4), which is related to the increased incidence of DT104 in human infection in 2008 compared to previous years.
The relatively small number of the isolates per year and the differences in proportion of multi drug resistant phage types per category and per year affect the trend analysis.

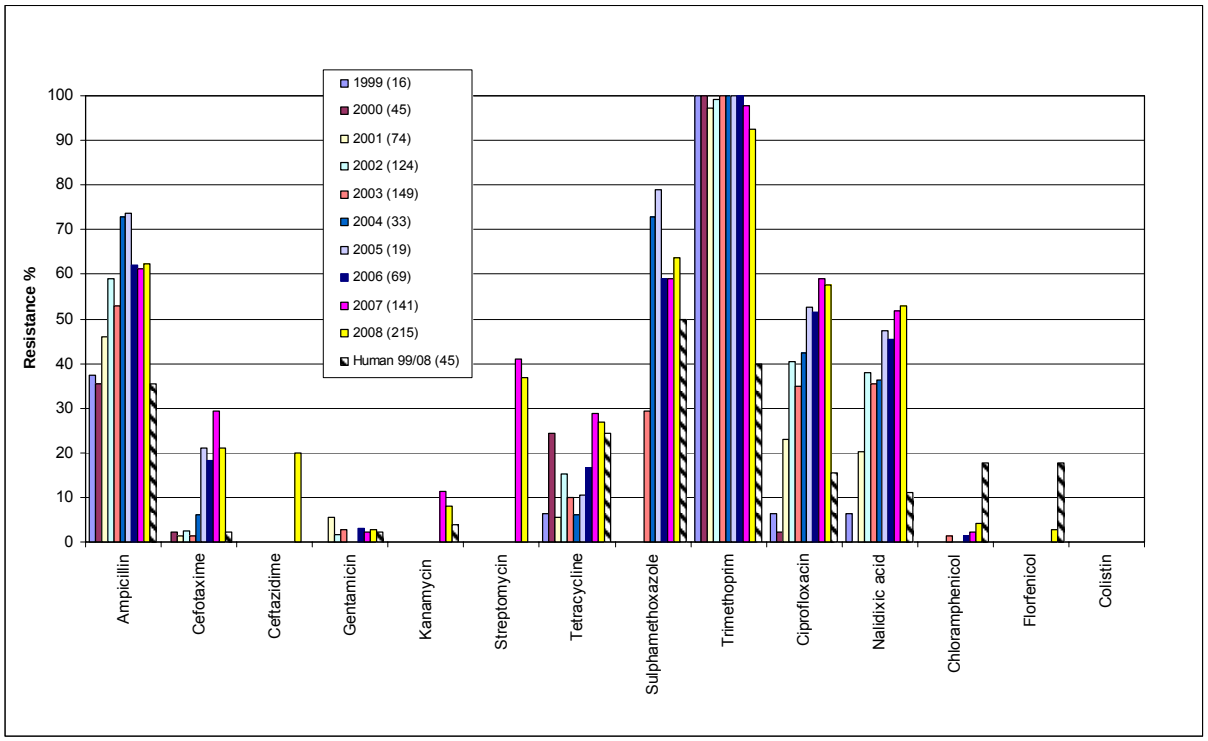
Figure 6.4. Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals from 1999 – 2008.



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

S. Java was in 2008 still the most predominant serovar isolated in broiler production. This is confirmed by the isolation rate of 76% of this serovar from poultry products in 2008.
In 2008 9 *S.* Java were isolated from a human infections. These were all trimethoprim susceptible and therefore not related to the clone spreading in Dutch poultry and probably travel related. However, two of these isolates were reduced susceptible to ciprofloxacin (MIC 0.5 mg/L), but not high level resistant against nalidixic acid (MIC 32 mg/L). This quinolone resistance pattern is typical for plasmid mediated quinolone resistance genes (*qnr*). From poultry, 195 strains were isolated of which 93% harbored the phenotype typical for the clone, which is slightly less that previous years (98%).
Non-wild type susceptibility to ciprofloxacin occurred in 60% of *S.* Java isolated from poultry (N = 117). In 2008, 12 isolates were found with high level resistance to ciprofloxacin (MIC 2 – 16).
Resistance to cefotaxime (ESBL-producers) was 21% in all isolates and 23% in isolates from poultry. This is related to the increase in ESBLs in commensal *E. coli* from broilers since 2003, by horizontal transfer of plasmid mediated beta-lactamases. Third-generation cephalosporins are not used in broiler production, but the use of ceftiofur in combination with Marek vaccine or with in ovo vaccination is a common off-label use procedure in the poultry reproduction and breeding sectors. It is likely that this has contributed to selection and vertical transmission of ESBLs in the poultry production pyramid as was recently described to occur in Canada (Dutil et al., Emerging Infectious Diseases 2010 Jan;16(1):48-54).

Figure 6.5. Trends in resistance (%) of *S.* Paratyphi B var. Java isolated from poultry from 1999 – 2008 and humans (Grey-white dashed bars indicate all humans *S.* Java isolates from 1999 – 2008).



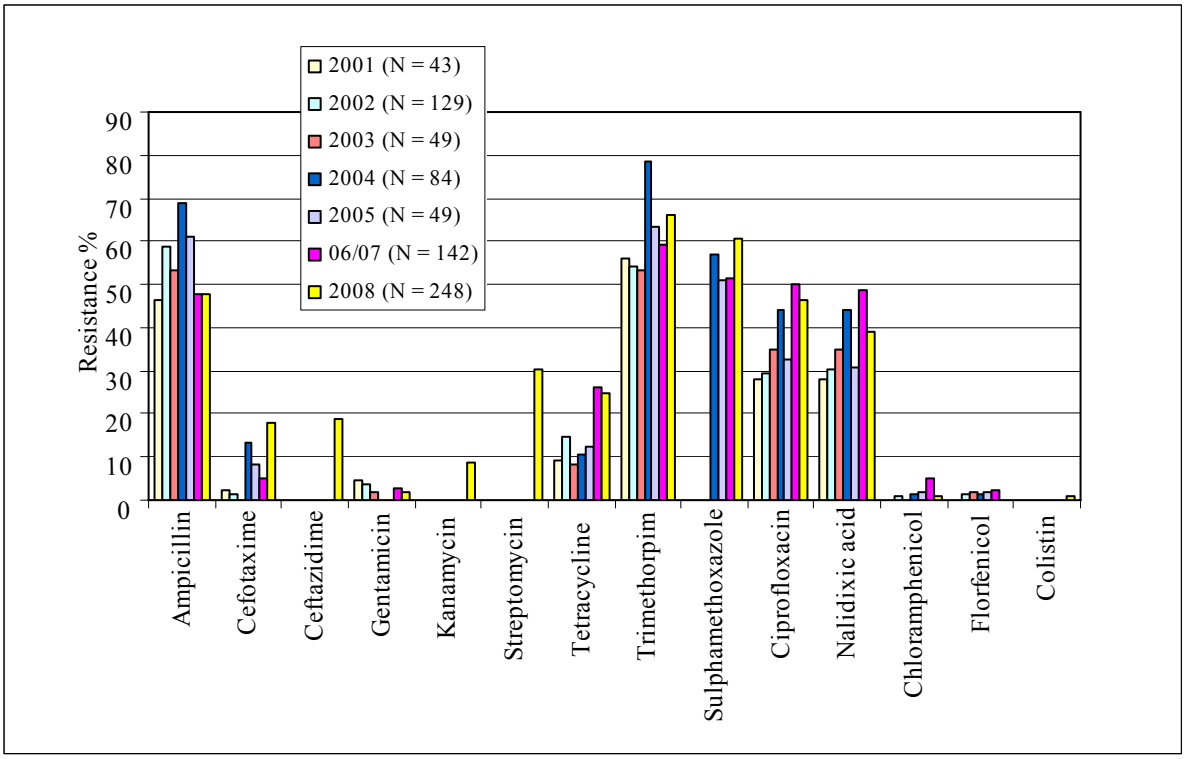
Salmonella in raw meat products of food-animals at retail

Table 6.6. Resistance (%) of *Salmonella enterica* isolated from raw meat from poultry, and other raw meat sources in 2008.

	Poultry *S*. Java N = 153	Poultry other serovars N = 95	Other raw meat sources N = 61
Ampicillin	60.8	49.5	44.3
Cefotaxime	18.3	10.5	14.8
Ceftazidime	19.0	7.4	21.3
Gentamicin	2.6	2.1	1.6
Kanamycin	8.5	6.3	4.9
Streptomycin	37.9	24.2	29.5
Tetracycline	21.6	25.3	50.8
Sulfamethoxazole	66.7	68.4	78.7
Trimethoprim	97.4	51.6	26.2
Ciprofloxacin	56.2	31.6	23.0
Nalidixic acid	50.3	27.4	14.8
Chloramphenicol	1.3	6.3	18.0
Florfenicol	0	5.3	11.5
Colistin	0.7	1.1	1.6

In 2008 in raw meat products originating from poultry *S*. Java was still by far the most prevalent serovar isolated (Table 6.7). Resistance levels for the quinolones and cephalosporins are similar as observed in isolates from broilers (Table 6.6, Figure 6.6).

Figure 6.6. Trends in resistance (%) of *Salmonella enterica* subspecies *enterica* isolated from poultry products in the Netherlands from 2001 – 2008.



Resistance trends are presented for poultry products only, because in beef and pork the numbers of isolates examined are too small to provide an accurate estimate (Figure 6.6). The variable contribution of *S*. Java to the annual resistance percentages over all serotypes hampers the interpretation of the observed trend in the resistance.

Table 6.7. Distribution of *Salmonella* serovars, in poultry meat at retail (Surveillance data of Food and Consumer Product Safety Authority (VWA) from 1997 – 2008.

	1997	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**
N	1314	1077	859	1454	1578	1600	1510	1482	1474	1539	1403	1505
Salmonella spp. positive (%)	29.1	20.2	17.6	21	16.3	13.4	11.3	7.4	9.4	8.4	8,1	8,1
	Main serovars as a fraction of all isolates (%)											
Paratyphi B Java	15	11.4	13.9	33.1	43.2	53.5	45.6	58.2	46.8	38.5	59,6	76,2
Enteritidis	20.2	12.8	26.4	6.6	8.2	2.3	8.8	5.5	7.2	6.6	2,0	1,6
Hadar	10.1	6.1	4.5	3.3	4.2	0.9	1.8	-	1.4	5.7	1,0	2,5
Indiana	6.1	8.3	9.3	10.2	11.6	6.5	6.4	1.8	2.2	4.1	6,1	0,8
Infantis	9.2	5	3.6	6.6	7	7.9	11.7	-	11.5	13.9	13,1	4,9
Virchow	4.6	2.8	2.6	10.2	3.5	5.6	5.8	4.5	8.6	11.5	4,0	1,6
Typhimurium	7.8	3.6	1.3	0.1	7.4	7.4	5.8	3.6	5	1.6	1,0	0,8
(DT104)	(..)	(1.8)	(0.7)	(0.1)	(7)	(2.8)	(5.3)	(..)	(2.2)	(..)	(..)	(..)
Corvallis									4.3	1.6		
Other types	27	53.6	39.7	30	22.3	23.3	19.9	26.4	13	16.5	13,2	11,6

Table 6.7 presents the contamination rates of *Salmonella* in poultry meat products over the years. The tendency to decrease stopped in 2006, after which it remained stable at 8%. The fraction *S*. Enteritidis however is still decreasing. In organic poultry meat the contamination rate was approximately 12% (monitoring program of the Food and Consumer Product Safety Authority). The contamination rates of *S*. Java continued to increase, while the rates for Typhimurium and Enteritidis remained low.

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

Table 6.8 presents the most prevalent serovars found in animal feeds from 2001 – 2008 per single and or compound feed type. Additionally, R% of Salmonella strains isolated from incidental animal sources are presented. The serotypes Senftenberg, Agona, Mbandaka, Lexington and Rissen are most frequently isolated from animal feeds**.** Resistance in these serovars is uncommon compared to isolates from animals or human sources except tetracycline resistance. Resistance against cefotaxime is present in isolates from soy and compound feeds.
In salmonella's isolated from turkeys, horses and ducks, more resistance was observed than in strains from pigeons or reptiles**.** Nalidixic acid and ciprofloxacin resistance was highest in turkeys and ducks, animals with a substantial consumption of quinolones (only fluoroquinolones are licensed for use in Turkeys). Cephalosporin resistance was also observed in isolates from turkeys

Table 6.8. The most prevalent serovars isolated from animal feed and resistance (R%) of isolates of *Salmonella enterica* per single and or compound feed type in 2001 – 2008. Additionally, R% of *Salmonella* strains isolated from incidental animal sources over 2001 – 2008 are presented.

			Animal feed (or ground substance)								Animals				
			Fish meal (56)	Animal meal (67)	Soy (feed, N=999)	Rapeseed (feed, N=359)	Single feed, other (361)	Composite feed (151)	**Feed 2007-2008, N=282**	Feed 2001-2006, N=1711	Turkey (100)	Horse (45)	Duck (20)	Pigeon (45)	Reptilian/Amfibian (69)
Serovar	**N**	**Antibiotics**	% resistant isolates 2001-2008						**%R**	%R	% resistant 2001-2008				
Senftenberg	255	**ampicillin**	0	1,5	0,5	0,8	1,7	1,3	**1,1**	0,8	43,0	20,0	20,0	11,1	1,4
Agona	211	**cefotaxime**	0	0	0,2	0	0	0,7	**0,7**	0,1	2,0	0	0	0	0
Mbandaka	183	**cefuroxime**	0	0	0	0	0	0	--	0	0	0	--	0	0
Lexington	169	**ceftazidime**	0	0	0,2	0	0	0	**0,4**	0	2,7	0	0	0	--
Rissen	132	**gentamicin**	0	0	0	0	0,3	0	**0,0**	0,1	13,0	0	0	0	0
Cubana	125	**kanamycine**	--	--	7,5	--	16,7	--	**8,8**	--	66,7	--	--	--	--
Livingstone	93	**neomycin**	0	2	0,5	0	1,7	0	**4,3**	0	15,0	0	0	0	0
Tennessee	85	**streptomycin**	--	--	7,5	--	8,3	--	**5,8**	--	55,6	--	--	--	--
Anatum	77	**tetracyclin**	0	3,0	0,9	0,3	1,7	4,6	**1,1**	1,3	42,0	17,8	5,0	6,7	2,9
Havana	64	**trim/sulpha**	0	0	0,5	0	0,8	0	--	0,4	0	33,3	--	0,0	0,0
Kentucky	56	**sulphamethoxazole**	0	0	0,5	2,0	1,7	5,7	**1,8**	1,3	36,9	11,1	0	9,1	--
Oranienburg	41	**trimethoprim**	0	1,5	0,4	0,8	0,3	3,3	**1,4**	0,6	12,0	17,8	5	0	0
Montevideo	37	**ciprofloxacin**	1,8	1,5	0,2	0	1,1	2,6	**1,1**	0,5	46,0	2,2	15,0	0	0
Infantis	36	**naladixic acid**	0	1,5	0,1	0	0,8	2,0	**0,7**	0,4	39,0	2,2	10,0	0	0
Minnesota	34	**chloramphenicol**	0	1,5	0,7	0,8	1,9	2,6	**1,8**	1,0	6,0	15,6	5	11,1	1,4
Cerro	32	**florfenicol**	0	0	0,5	0,8	0,8	0	**1,1**	0,5	4,0	6,7	0	11,1	1,4
Yoruba	29														

17 main serotypes	1659 (83%)
All serotypes	1988

For The *Salmonella* isolates from turkeys the MIC-values for streptomycin, kanamycin, streptomycin and sulphamethoxazole were determined for a subset of the isolates.

Campylobacter spp.

Highlights
In *Campylobacter* resistance against the (fluoro)quinolones continues to increase in 2008 in isolates from animals and from humans. In 2008 approximately 50% of the *Campylobacter* strains isolated from humans were resistant against ciprofloxacin, compared to 35% in the period 2002-2005. More than 60% of the isolates from broiler chickens were ciprofloxacin resistant compared to 35-45% in 2002 - 2005.
High levels of resistance were also observed for tetracycline, while resistance against the macrolides (erythromycin, tulathromycin and clarithromycin) remains at a fairly low level.
In general, *C. coli* showed much more resistance and at higher levels than *C. jejuni*.

Table 6.9 presents the MIC-distributions and resistance percentages for all *Campylobacter jejuni* and *C. coli* strains isolated from broilers, pigs, cattle and veal calves in 2008. In *C. jejuni* highest resistance levels are observed for tetracycline and the quinolones.

In *Campylobacter* resistance against quinolones is still increasing. In 2008, 55.6% of all *C. jejuni* strains isolated from animals were resistant against ciprofloxacin (Table 6.9). In isolates from poultry, which is considered to be a more important source for human infections than isolates from cattle or pigs, the ciprofloxacin resistance levels are 57.1% in isolates from poultry meat and 66.3% in isolates from broiler faeces.
Also in *C. coli* from broilers the levels of resistance against quinolones show a tendency to increase (Figure 6.7). In contrast, in *C. coli* from pigs quinolone resistance levels are fairly stable at 5-10%, reflecting the limited usage of quinolones in pigs. Also in isolates from human infections an increase in fluoroquinolone resistance is observed from 45% in 2007 to 50% in 2008 (Figure 6.8, Table 6.11).

Table 6.9. MIC distribution (in %) for all *C. jejuni* (N=126, of which 90 from broilers, 10 from dairy cows, 4 from pigs and 22 from veal calves) and *C. coli* (N=191, of which 119 from pigs, 15 from broilers, 5 from dairy cows and 52 from veal calves) isolated from broiler-chickens, pig and cattle faeces in the Netherlands in 2008.

C. jejuni	MIC (%) distribution mg/L															
(N = 126)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin		1.6	0.8	0.8	6.3	31.0	31.7	7.1	4.8	15.9						27.8
Gentamicin		83.3	9.5	5.6				0.8		0.8						1.6
Neomycin			92.1	6.3	0.8			0.8								1.6
Streptomycin				90.5	0.8	0.8		4.0	0.8			3.2				8.7
Tetracycline			32.5	6.3	1.6	0.8		0.8	0.8	11.9	45.2					59.5
Sulphamethoxazole							3.2	5.6	15.9	16.7	37.3	13.5	0.8	3.2	4.0	7.9
Ciprofloxacin	31.7	11.1	0.8	0.8	0.8	3.2	20.6	19.8	11.1							55.6
Nalidixic acid					4.0	23.8	11.9	2.4	0.8	0.8	15.9	40.5				57.9
Erythromycin			40.5	36.5	15.9	1.6	0.8		0.8		4.0					5.6
Clarithromycin			20.6	54.0	13.5	7.1	0.8			0.8	3.2					4.0
Tulathromycin			73.0	19.8	3.2		0.8			0.8	2.4					3.2
Chloramphenicol					43.7	38.1	15.9	1.6	0.8							0.8

C. coli	MIC (%) distribution mg/L															
(N = 191)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin				1.0	2.1	18.8	28.3	32.5	4.2	13.1						17.3
Gentamicin		37.2	37.7	22.5						2.6						2.6
Neomycin			49.7	26.2	11.5	1.6			1.6	2.1	7.3					12.6
Streptomycin				19.9	6.3	0.5	0.5	17.3	34.6	8.9	3.1	8.9				73.3
Tetracycline			7.9	7.3	2.6	1.6		0.5	1.6	3.7	74.9					82.2
Sulphamethoxazole							7.9	13.1	17.3	9.9	2.1	0.5	15.2	25.1	8.9	49.2
Ciprofloxacin	46.6	15.2	3.1			1.0	11.0	19.4	3.7							35.1
Nalidixic acid						17.3	39.8	7.9		1.0	16.8	17.3				35.1
Erythromycin			6.3	16.8	29.3	26.7	3.1	1.0		1.0	15.7					16.8
Clarithromycin			4.2	12.6	26.7	29.3	9.4	1.0	1.0	0.5	15.2					15.7
Tulathromycin			51.3	14.7	15.7	1.6		0.5	1.0	8.4	6.8					16.2
Chloramphenicol					9.9	35.1	48.7	5.2	1.0							1.0

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

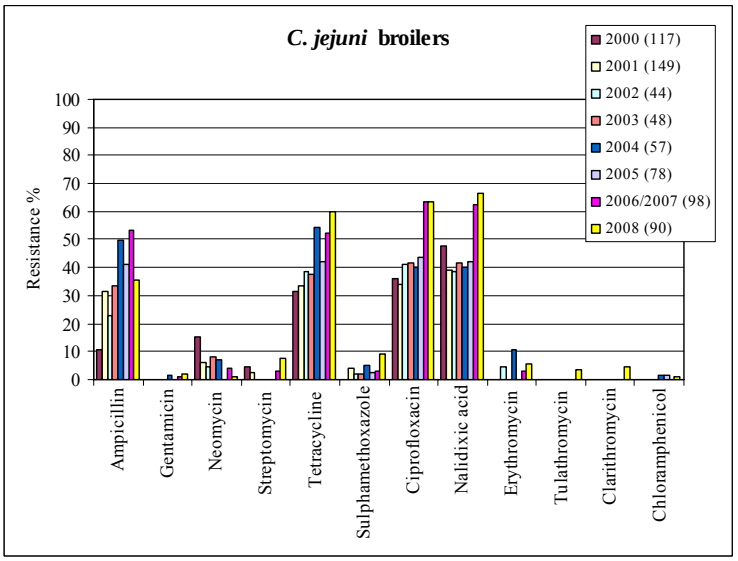
Macrolide resistance remains low in *C. jejuni*. In 2008, 5.6% of the *C. jejuni* strains were resistant against erythromycin. Five *C. jejuni* strains were detected with an MIC of 128 mg/L for erythromycin, of which four were from broiler chicken faeces and one strain was isolated from a veal calf. During 2007 the related macrolides clarithromycin and tulathromycin (a triamilide antibiotic) have been included in the test panel. All but one out of 90 erythromycin resistant chicken strains showed high levels of resistance against these two antibiotics as well.
In *C. coli* resistance against macrolides occurred more frequently compared to *C. jejuni*, with similar levels for erythromycin, clarithromycin and tulathromycin. In *C. coli* from pigs, erythromycin levels seem to have increased again in 2006/2007 after an initial decrease from 2000 to 2005 (fig 14) which was explained by the ban of tylosin as growth promoter in 1999. The current increase may be related to increased therapeutic usage of macrolides (tylosin, tilmicosin, tulathromycin) in pigs, since 2005.

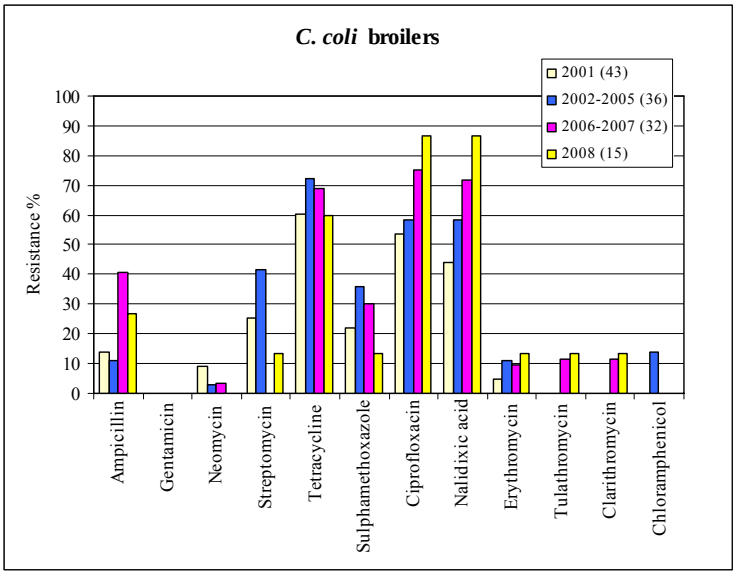
Table 6.10. Resistance percentages of *C. jejuni* and *C. coli* isolated from raw meat products from poultry and from faecal samples of broilers, veal calves, dairy cows (only *C. jejuni*) and pigs (only *C. coli*) in 2008.

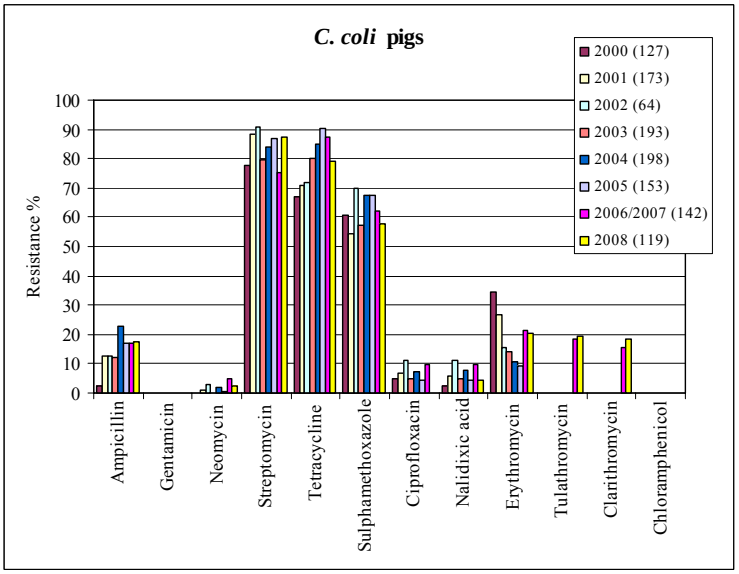
	C. jejuni				*C. coli*			
	poultry products	**broilers**	**veal calves**	**dairy cows**	**poultry products**	**broilers**	**veal calves**	**pigs**
N	359	90	22	10	45	15	52	119
Ampicillin	44.0	35.6	9.1	0	28.9	26.7	13.5	17.6
Gentamicin	0.3	2.2	0	0	0	0	9.6	0
Neomycin	3.3	1.1	4.5	0	6.7	0	40.4	2.5
Streptomycin	5.6	7.8	9.1	0	26.7	13.3	63.5	87.4
Tetracycline	48.2	60.0	72.7	20.0	71.1	60.0	98.1	79.0
Sulphamethoxazole	6.7	8.9	4.5	0	20.0	13.3	42.3	58.0
Ciprofloxacin	57.1	63.3	45.5	20.0	55.6	86.7	86.5	4.2
Nalidixic acid	63.5	66.7	45.5	20.0	57.8	86.7	86.5	4.2
Erythromycin	4.2	5.6	9.1	0	20.0	13.3	11.5	20.2
Tulathromycin	2.5	3.3	4.5	0	20.0	13.3	11.5	19.3
Clarithromycin	3.9	4.4	4.5	0	20.0	13.3	11.5	18.5
Chloramphenicol	0.6	1.1	0	0	0	0	3.8	0

Resistance data in various animal species reflect the application of antibiotics of choice in different animal sectors (Table 6.10). The quinolone resistance levels are highest in veal calves and broilers, the animal species in which this drug class is used predominantly. For broilers, samples from caecal contents as well as samples from carcasses and meat products are shown in Table 6.10. In *C. jejuni* the resistance levels in isolates from broiler faeces and from poultry raw meat products sampled by the Dutch Food Safety Authority at retail are very similar, while in *C coli* resistance levels against fluoroquinolones in poultry products is lower compared to live animals.

Figure 6.7. Trends in resistance (%) of *C. jejuni* (isolated from broilers) and *C. coli* (from broilers and slaughter pigs) from 2000 - 2008 in the Netherlands.







In *Campylobacter* spp. isolated from humans trends in increase of resistance against fluoroquinolones are similar as observed in isolates from broiler chickens. Resistance against tetracyclines varies around 20%. Resistance against erythromycin remains stable at a low frequency (Figure 6.8 and Table 6.11).

Figure 6.8. Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2008 at the regional Public Health Laboratories (PHLS) of Arnhem and Heerlen covering 990.000 inhabitants (400-700 isolates per year). The continuous line represents national surveillance data from 2002 onwards; the average number of strains tested per year was approximately 2400, ranging from 1900 – 2900.

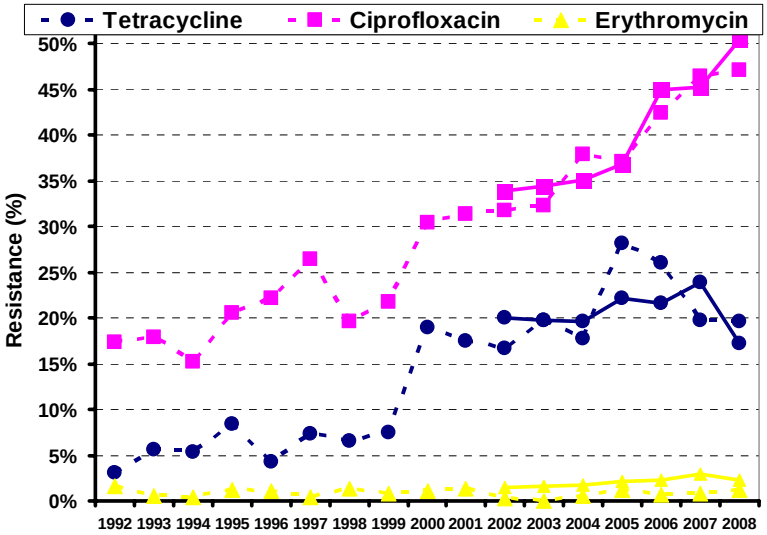


Table 6.11. Percentage Campylobacter jejuni and C. coli isolates from humans resistant against fluoroquinolones, tetracycline and erythromycin from 2002 to 2008.

	Percentage of resistant isolates			
Campylobacter spp.	2002-2005	2006	2007	2008
Fluoroquinolone	35,2	45	45,2	50,5
Tetracycline	20,2	21,7	23,9	17,2
Erythromycin	1,5	2,2	2.9	2,3

Table 6.12 shows that similar as in previous years, in travel-related infections fluoroquinolone resistance occurred more frequently than in isolates from domestically acquired infections. For tetracycline this difference was observed for *C. jejuni* only.

Table 6.12. Domestically acquired and travel related resistance in *C. jejuni* and *C. coli* isolated from humans from 2002 - 2008 from all 16 PHLS covering > 50% of the Dutch population.

	2002-2005								2006-2008							
	Domestically acquired				Travel related				Domestically acquired				Travel related			
	C. jejuni		*C. coli*		*C. jejuni*		*C. coli*		*C. jejuni*		*C. coli*		*C. jejuni*		*C. coli*	
	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	6792	**32,7**	386	**36,3**	600	**53,5**	56	**50**	6692	**45,4**	495	**45,1**	345	**62,9**	35	**65,7**
Tetracycline	5028	**18,5**	353	**22,7**	425	**27,1**	49	**20,4**	4482	**19,8**	393	**25,4**	169	**27,8**	32	**18,8**
Erythromycin	5735	**1,2**	372	**3**	511	**1,6**	52	**0**	5361	**1,9**	430	**4,7**	265	**3,4**	32	**9,4**

In the surveillance program carried out by the Dutch Food and Consumer Product Safety Authority (VWA), meat products are tested for the presence of zoonotic food pathogens. Data for *Campylobacter* are shown in Table 6.13. After a decline in 2006 and 2007, levels in poultry products are again comparable to the levels in previous years.

Table 6.13. Isolation rates of *Campylobacter* spp, strains in poultry products at retail, from 1996-2008.

	1996	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**
N	1325	1314	1077	859	1454	1581	1604	1431	1477	1404	1473	1404	1515
% *Campylobacter* spp.	36.2	31.8	26.9	23.5	30.5	32.5	31.3	25.9	29.3	22.1	14.2	15.4	23.6
(organic)	(n.a.)	(n.a.)	(n.a.)	(n.a.)	(n.a.)	(n.a.)	(n.a.)	(36.3)	(43.9)	(33.3)	(29.8)	(n.a.)	(±12%)

n.a. not analysed

Shigella toxin producing E. coli *O157*

Highlights
In previous years, resistance levels in human isolates of *E. coli* O157 have been fairly low, while resistance in isolates from calves was more commonly present. In 2008, resistance levels of human isolates show a tendency to increase, resembling the resistance profiles in cattle.

In 2008, 208 Shiga-toxin producing *E. coli* O157 (STEC) isolates were tested for susceptibility. Isolates were obtained from human patients (N = 47) and from cattle specimens (N = 161). The majority of the cattle isolates originated from faecal samples of healthy veal calves (N = 143).
MIC results are shown in Table 6.14. Traditionally, resistance in *E. coli* O157 from human specimens is very low, while in cattle strains, resistance occurs more commonly, with levels up to 20%. In 2008 however, resistance profiles in human and cattle strains are very similar. Highest rates of resistance were against sulphamethoxazole (17% and 21.7% in humans and cattle resp.) and streptomycin (19.1% and 12.4% resp.).
Based on MIC profiles, no ESBL suspect phenotypes were present as all isolates were susceptible to cefotaxime and ceftazidime. All isolates were also susceptible to ciprofloxacin, nalidixic acid and colistin.

Although resistance rates in previous years showed considerable variation, in the past years resistance in animal isolates showed a tendency to increase, particularly against ampicillin and sulphamethoxazole. Based on the data from 2008, resistance levels seem to be stable in cattle isolates compared to 2006/2007. In contrast to this, the resistance profiles in human isolates tend to increase, more resembling the situation in cattle.

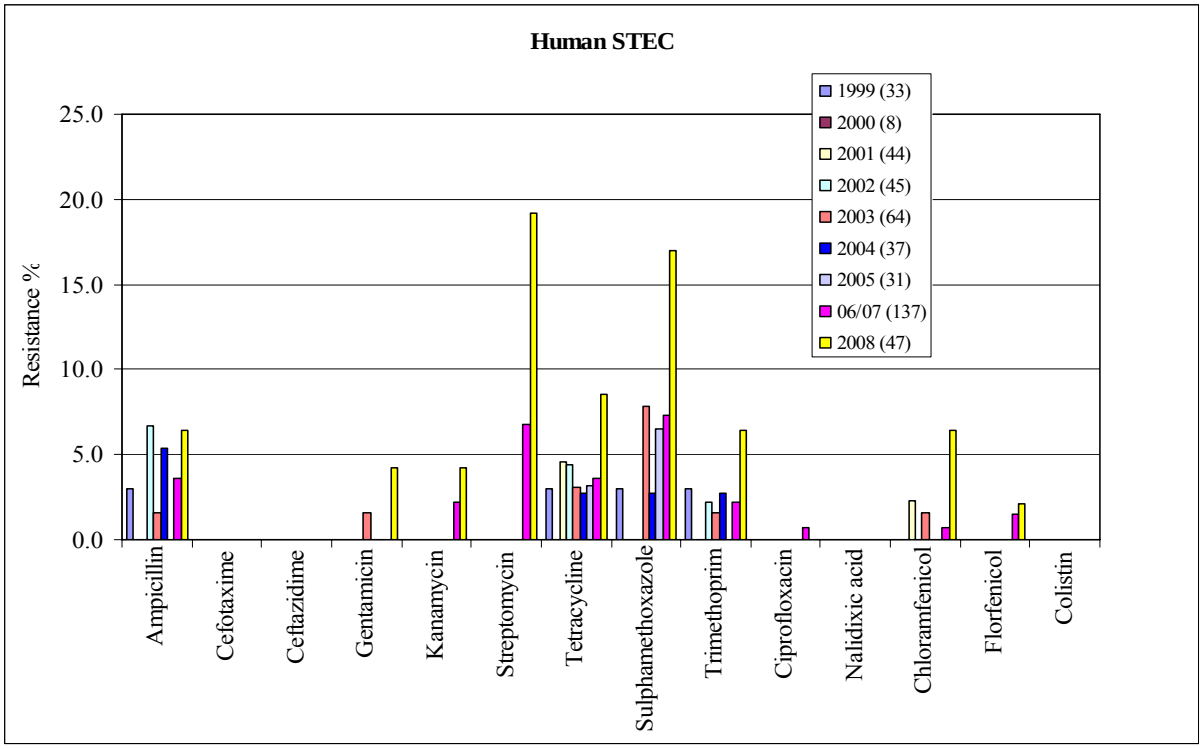
Table 6.14. MIC distribution (in %) for *E. coli* O157 isolated in the Netherlands from human (N=47) and cattle faeces (N=161) in 2008.

Human	MIC (%) distribution mg/L																		
N=47	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin							2.1	12.8	78.7				6.4						6.4
Cefotaxime			95.7	4.3															0
Ceftazidime					100														0
Gentamicin						46.8	46.8	2.1		2.1			2.1						4.3
Kanamycin									95.7						4.3				4.3
Streptomycin									17.0	63.8			2.1	6.4	10.6				19.1
Tetracycline								80.9	10.6					8.5					8.5
Sulphamethoxazole										83.0								17.0	17.0
Trimethoprim						93.6							6.4						6.4
Ciprofloxacin	78.7	21.3																	0
Nalidixic acid									100										0
Chloramphenicol									2.1	87.2	4.3		2.1	4.3					6.4
Florfenicol									10.6	85.1	2.1			2.1					2.1
Colistin										100									0

Cattle	MIC (%) distribution mg/L																		
N=161	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%
Ampicillin								6.8	81.4	3.1			8.7						8.7
Cefotaxime			94.4	5.6															0
Ceftazidime					96.3	3.7													0
Gentamicin					2.5	62.7	32.3	2.5											0
Kanamycin									90.7	6.8	1.2				1.2				2.5
Streptomycin								0.6	6.2	78.3	2.5				12.4				12.4
Tetracycline								50.3	41.6				0.6	7.5					8.1
Sulphamethoxazole										58.4	18.0	1.9					1.9	19.9	21.7
Trimethoprim						88.8	2.5						8.7						8.7
Ciprofloxacin	49.7	47.8	2.5																0
Nalidixic acid									99.4	0.6									0
Chloramphenicol								1.9	82.0	11.8	0.6			3.7					5.0
Florfenicol								3.1	30.4	60.9	0.6			5.0					3.7
Colistin										100									0

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

Figure 6.9. Trends in resistance percentages of *E. coli* O157 (STEC) isolated in The Netherlands from 1998 – 2008.



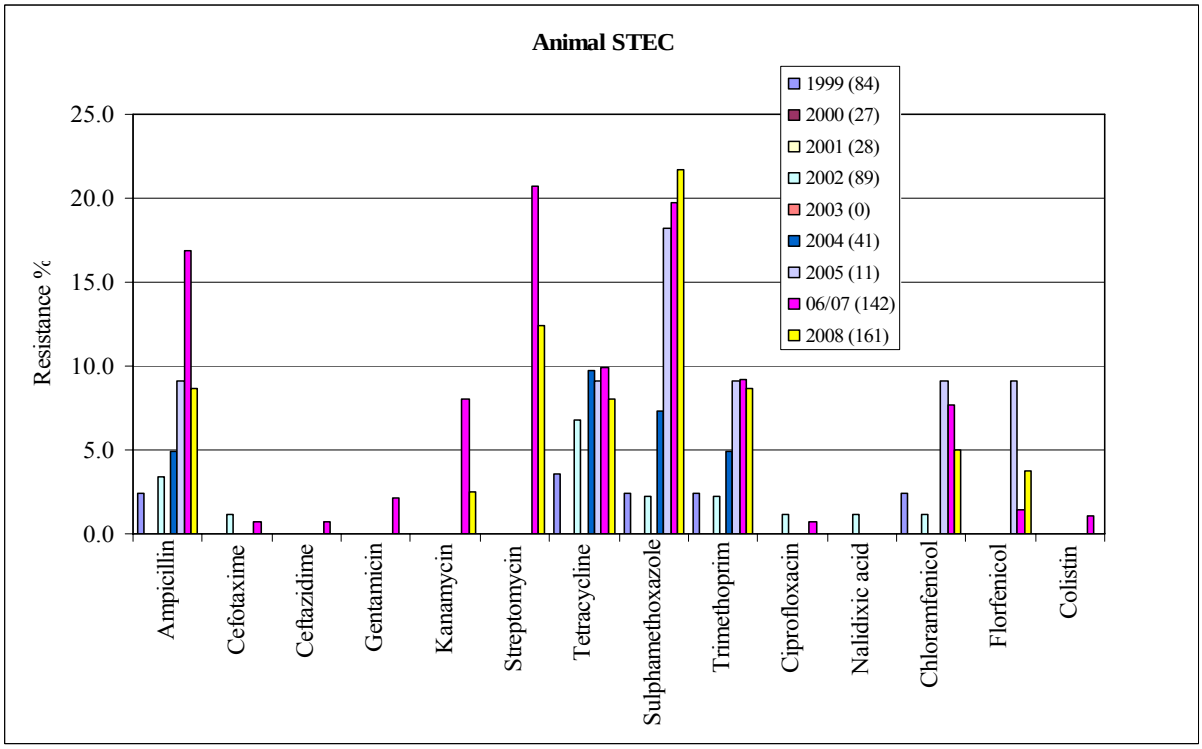
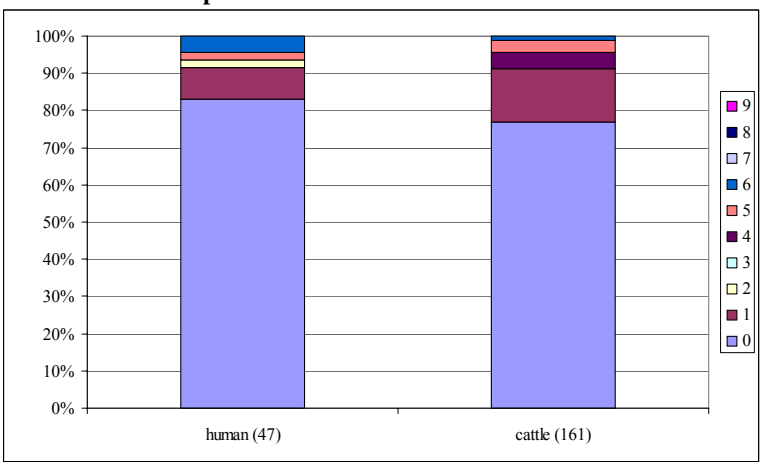


Figure 6.10. Percentages of E. coli O157 strains fully susceptible, resistant to one to a maximum of nine antimicrobial classes from human and cattle specimens in 2008.



Information on multiple drug resistance is shown in Figure 6.10. Concerning human isolates, in 6% of the strains resistance to five or six classes of antibiotics was observed, while 9% of the cattle strains showed resistance against four or more antibiotic classes.

7. Commensal indicator organisms

The level of antimicrobial resistance in commensal organisms isolated from samples taken from the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics 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.
From 2005 onwards, resistance in isolates from both dairy cattle and veal calves has been monitored using samples that were taken at farms to determine the prevalence of *Salmonella*, *E. coli* O157 and *Campylobacter*.
Resistance percentages in Table 7.1 and Table 7.3 indicate the level of resistance in all *E. coli* and in *E. faecium* and *E. faecalis* strains of slaughter pigs, broilers, dairy cows and veal calves, respectively. The sampling strategy implies that this method is inherently insensitive for detecting resistance as only one randomly selected isolate is tested from a single sample taken from one animal per epidemiological unit (herd or flock). The total sample of selected isolates is intended to represent the *E. coli,* or *Enterococcus* species population of each animal species of the entire Netherlands. One percent resistance in e.g. *E. coli* indicates that in all animals 1% of the *E. coli* bacteria are resistant. Because each animal harbours about 10^6 cfu/g faeces *E. coli* in its gut, 1% would be approximately 10^4cfu/g faeces. This means that the absence of resistance in these datasets does not exclude the possibility that resistance is present in small numbers in each animal.

Escherichia coli

Highlights
Resistance rates in *E. coli* continue to increase in slaughter pigs, broiler chickens and dairy cows. Also in veal calves resistance is high, but for most antibiotics that were tested, rates seem to either stabilize or show a moderate decrease.
In broiler chickens, resistance against the quinolones is again disturbingly high and moreover still increasing. In 2008, more than 60% of all isolates were resistant against nalidixic acid and ciprofloxacin compared to almost 50% in 2006/2007.
Another matter of concern is the emergence of extended spectrum beta-lactamases (ESBL) in broilers chickens since 2003. In 2008, approximately 15% of the *E. coli* isolates from these chickens were resistant against cefotaxime and ceftazidime, indicative of the frequent presence of ESBL's.
Multidrug resistance is increasing in all animal species tested with highest levels in veal calves and broilers. In dairy cattle, resistance has been traditionally low, but is increasing alarmingly fast.

Table 7.1 presents the MIC-distributions and resistance percentages for *E. coli* strains isolated from slaughter pigs, broilers, veal calves and dairy cattle in 2008. In isolates from all species included in this survey, highest resistance levels are observed against ampicillin (ranging from 9.5% in dairy cows to 65.5% in broiler chickens), streptomycin (ranging from 10.1% in dairy cows to 60% in broiler chickens), tetracycline (13.5% in dairy cattle to 67.9% in slaughter pigs), sulphamethoxazole (10.1% in dairy cattle to 70.9% in broiler chickens) and trimethoprim (6.1% in dairy cattle to 60% in broiler chickens).
The increasing quinolone resistance is a disturbing trend. In broiler chickens over 60% of all isolates that were tested showed non-wild type susceptibility[6] to nalidixic acid and ciprofloxacin. High level resistance (MIC >1 mg/L) against ciprofloxacin in broiler chickens was on a similar level as in 2006/2007 (6.3% of the isolates). Also in veal calves non-wild type susceptibility to quinolones was frequently observed (20.3%), 9.1% of which were high level resistant against ciprofloxacin. Quinolone resistance was lowest in slaughter pigs (<2%).
Another worrisome development is the frequent occurrence of ESBL-producing *E. coli*, especially in broiler chickens. ESBLs confer resistance to all beta-lactam antibiotics and is both a veterinary and a public health threat. In 2008, 15.0% of the isolates were non-wild type susceptible to cefotaxime and 14.5% to ceftazidime, thus potentially producing ESBL's. Although in 2007 the percentage of cefotaxime resistant isolates was more than 20%, in that year only 43 isolates were tested, while the resistance percentage in 2008 was based on 440 isolates and therefore a more precise estimate. This indicates that since 2005 the cefotaxime resistance levels are stable at approximately 15%. Also in pigs, calves and dairy cattle ESBL-suspected *E. coli* isolates are observed, although still at a relatively low level (<2%). In 2009 an ESBL-prevalence study was conducted on 26 poultry farms. On these farms from 25 - 41 animals fecal samples were taken and examined for the presence of ESBL-producing *E. coli* on MacConkey agar with 1 mg/L cefotaxime. The prevalence of ESBLs on these farms was very high, 100 % of investigated farms were positive for ESBL-producing *E. coli* and on 85% of these farms ≥80% (95% CI 71-99%) of the animals carried ESBL-producers in their faeces.

[6] a micro-organism is defined as wild type (WT) for a species by the absence of acquired and mutational resistance mechanisms to the drug in question. Wild type micro-organisms may or may not respond clinically to antimicrobial treatment (http://www.eucast.org).

Table 7.1. MIC distributions (in %) for *E. coli* isolated as indicator organism from intestines of slaughter pigs (N=296), broiler chickens (N=440), veal calves (N=153) and dairy cattle (N=148) in the Netherlands in 2008.

Slaughter pigs	**MIC (%) distribution mg/L**																		
N = 296	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**R%**
Ampicillin							1.7	18.6	40.5	3.7			35.5						35.5
Cefotaxime			86.5	12.5						1.0									1.0
Ceftazidime					93.2	5.4	0.3	1.0											1.4
Gentamicin					1.4	31.4	51.4	13.5	1.7	0.3	0.3								2.4
Kanamycin									64.9	27.7	5.7	0.7			1.0				7.4
Streptomycin									6.4	24.3	13.5	6.4	10.1	13.9	25.3				55.7
Tetracycline							2.0	16.6	12.8	0.7	0.7	0.3	14.2	52.7					67.9
Sulphamethoxazole										41.6	0.3	0.7			0.3		0.3	56.8	57.1
Trimethoprim						48.0	2.4	0.3					49.3						49.3
Ciprofloxacin	72.5	25.4	0.7		1.4														1.4
Nalidixic acid									95.9	1.4	0.7	0.3		1.7					2.0
Chloramphenicol								0.7	11.1	66.9	10.8	3.0	1.0	6.4					10.5
Florfenicol								2.0	18.2	72.3	6.4	1.0							1.0
Colistin										99.7	0.3								0.3

Broilers	**MIC (%) distribution mg/L**																		
N = 440	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**R%**
Ampicillin							0.7	8.6	20.5	4.8			65.5						65.5
Cefotaxime			62.7	20.9	1.4	0.2	0.7		0.2	13.9									15.0
Ceftazidime					78.2	7.3	3.0	4.8	2.0	1.6	2.0	1.1							14.5
Gentamicin						23.4	50.2	11.8	2.5	0.5	4.5	4.8	2.3						14.5
Kanamycin									51.1	27.3	4.8	1.1		0.2	15.5				21.6
Streptomycin									5.5	25.5	9.1	3.6	8.2	7.3	40.9				60.0
Tetracycline							3.6	16.4	20.7	1.1		0.2	8.0	50.0					58.2
Sulphamethoxazole										28.0	0.5		0.2	0.5		0.2	0.5	70.2	70.9
Trimethoprim						38.0	1.8	0.2					60.0						60.0
Ciprofloxacin	26.6	11.6	0.9	4.5	30.0	17.3	2.7	1.1		2.0	3.2								60.9
Nalidixic acid									36.8	1.4		1.4	3.9	56.6					61.8
Chloramphenicol									5.0	55.9	13.9	1.8	3.0	20.5					25.2
Florfenicol									11.1	67.5	16.6	2.3	0.2	2.3					4.8
Colistin										100									0.0

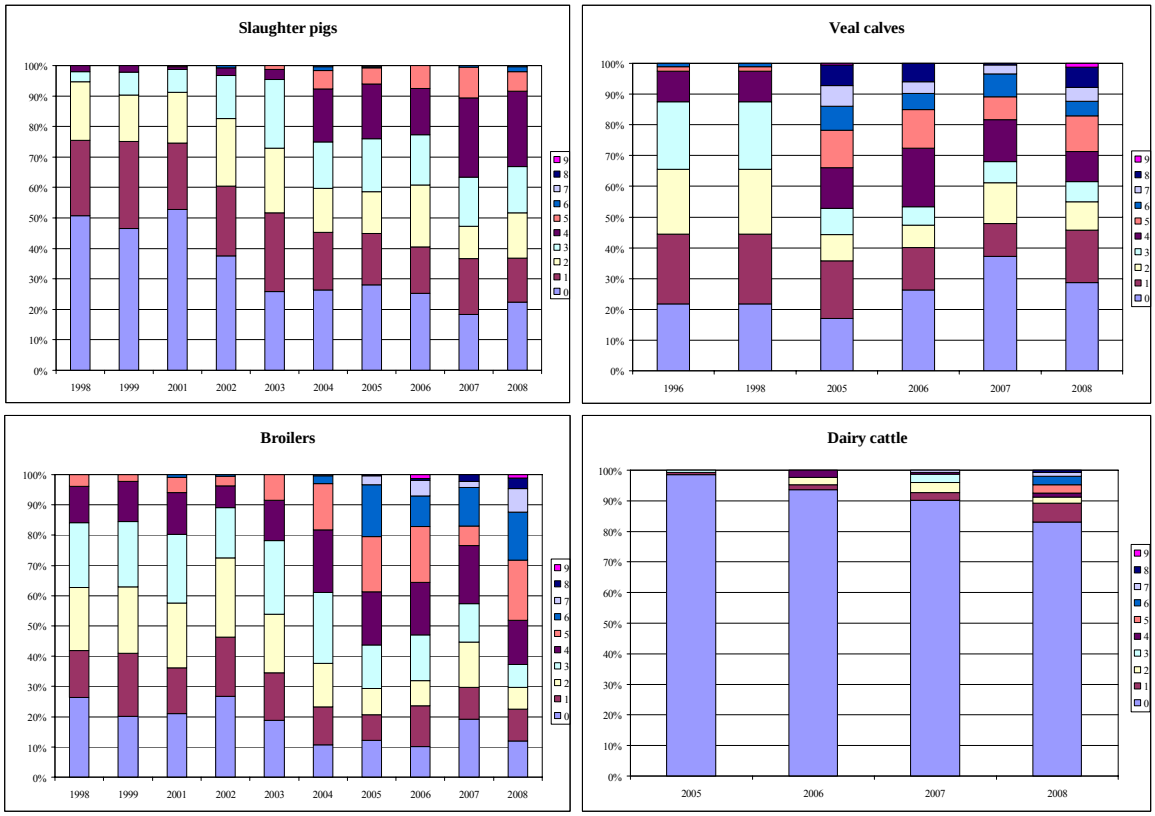
Veal calves	**MIC (%) distribution mg/L**																		
N = 153	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**R%**
Ampicillin							0.7	14.4	41.8	2.6			40.5						40.5
Cefotaxime			73.2	18.3	6.5		0.7	0.7		0.7									2.0
Ceftazidime					84.3	13.7	0.7	0.7		0.7									2.0
Gentamicin						30.7	47.1	9.8	2.0		2.0	2.6	5.9						12.4
Kanamycin									54.2	18.3	3.9	0.7	0.7	0.7	21.6				27.5
Streptomycin									11.1	36.6	7.8	1.3	4.6	9.2	29.4				44.4
Tetracycline							1.3	13.7	14.4	3.3		0.7	4.6	62.1					67.3
Sulphamethoxazole										53.6	0.7	0.7						45.1	45.1
Trimethoprim						56.9		0.7					42.5						42.5
Ciprofloxacin	60.1	15.7	3.9	0.7	4.6	5.2	0.7	2.6		1.3	5.2								20.3
Nalidixic acid									75.8	3.9				20.3					20.3
Chloramphenicol									3.9	60.1	17.0	1.3		17.6					19.0
Florfenicol									10.5	66.0	11.1	2.0	0.7	9.8					12.4
Colistin										99.3			0.7						0.7

Dairy cattle	**MIC (%) distribution mg/L**																		
N = 148	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**R%**
Ampicillin							2.0	16.2	66.9	5.4			9.5						9.5
Cefotaxime			79.1	18.9	0.7					1.4									1.4
Ceftazidime					93.2	5.4			0.7		0.7								1.4
Gentamicin					0.7	39.2	48.0	8.8	1.4		0.7	0.7	0.7						3.4
Kanamycin									73.6	19.6	2.0			0.7	4.1				6.8
Streptomycin									15.5	64.2	10.1	1.4	1.4	1.4	6.1				10.1
Tetracycline							8.1	27.0	49.3	2.0	0.7		1.4	11.5					13.5
Sulphamethoxazole										89.2	0.7							10.1	10.1
Trimethoprim						92.6	0.7	0.7					6.1						6.1
Ciprofloxacin	71.6	23.6		0.7	0.7	1.4	0.7			0.7	0.7								4.7
Nalidixic acid									93.9	0.7		0.7		4.7					5.4
Chloramphenicol									4.7	74.3	16.9		0.7	3.4					4.1
Florfenicol									8.8	78.4	10.1	0.7		2.0					2.7
Colistin										100									0.0

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

As in previous years, chloramphenicol resistance is frequently observed, in broilers (25.2%), veal calves (19%),to a lesser extent in pigs (10.5%) and dairy cattle (4.1%). Resistance rates to the related drug florfenicol, are much lower and primarily seen in veal calves. Although florfenicol is registered for use in both pigs and cattle for respiratory disease, the difference in resistance rates probably reflects the scale of use in these animals.
Although in pigs and veal calves, resistance rates are relatively stable over the years, in broiler chickens the chloramphenicol resistance rate has increased from 5% in 1998 to 25.2% in 2008 (Figure 7.2). This is remarkable as the use of chloramphenicol is prohibited for approximately 20 years, and an obvious selection pressure is absent. It is likely that chloramphenicol resistance is co-selected by other antimicrobial resistance genes.
In 2008, high level resistance against colistin was detected in an *E. coli* isolate from veal calves (MIC 64 mg/L). Colistin resistance is rare in Enterobacteriaceae. In the Netherlands colistin is commonly used in veal calves in combination with oxytetracycline as a so called starter mix, for prevention of bacterial infection during the first 10 days of the fattening period.

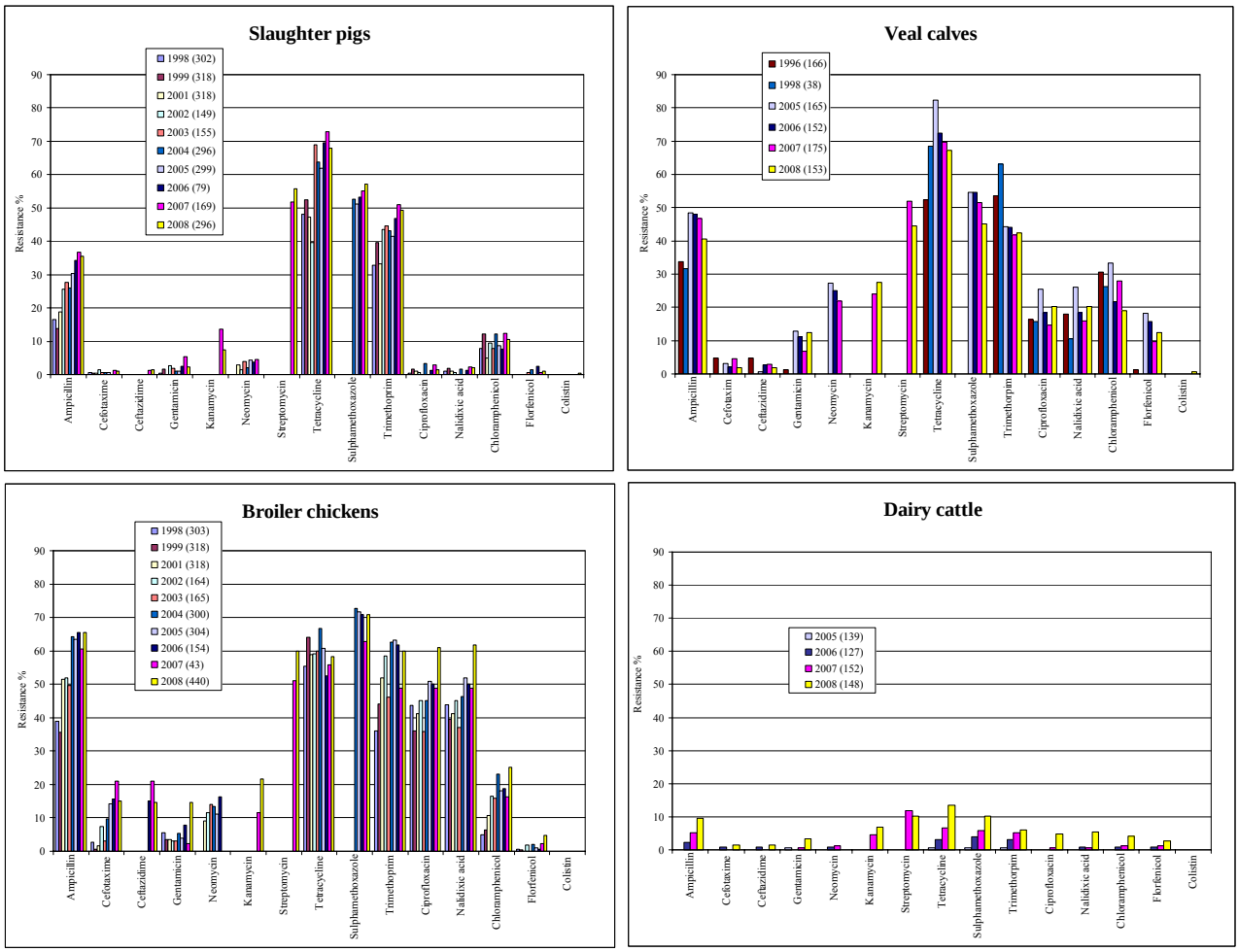
Figure 7.1. Trends in percentages of *E. coli* strains fully susceptible, resistant to one to a maximum of nine antimicrobial classes in broiler chickens, slaughter pigs and veal calves in the Netherlands from 1998 - 2008.



Multidrug resistance has increased since the start of the surveillance program in 1998 in all animal species tested, with highest levels in veal calves and broilers. However, since 2006 the levels remained stable in calves and broilers. From calves and broilers, strains were recovered with resistance against up to nine antibiotic classes. The random nature of the sampling method, in which each isolate represents a farm or flock of animals, indicates that such multidrug resistant strains are commonly present in these food animal species.
In slaughter pigs, the level of multidrug resistance is slightly lower. Still, multidrug resistance shows a tendency to increase. In 2008 resistance against seven classes of antibiotics was detected (0.3%) in a single strain, implicating the potentially widespread nature of such multidrug resistance in slaughter pigs as well.
In dairy cows, resistance has been traditionally low, but is increasing alarmingly fast in a few years time. In 2005, multidrug resistance was rarely observed, while in 2008 11% of the isolates were resistant to two or more antibiotic classes and resistance against up to eight classes was seen in individual isolates.

Except in broiler chickens and in dairy cattle the trends in resistance remained stable in 2008 compared to 2007 (Figure 7.2). In broilers the increase in resistance against ciprofloxacin is striking. It seems to reflect increased usage of quinolones in poultry. Similar trends are observed in *Salmonella* Java, and *Campylobacter* spp. from broilers.
In dairy cows for almost all antibiotics in the test panel an increase in resistance is observed. This is also a remarkable observation since in dairy cows antibiotics are mainly administered intramammary or in the uterus, which does not directly result in selection of resistance in the gastro-intestinal tract.

Figure 7.2. Trends in resistance (in %) of *E. coli* isolated from slaughter pigs and broilers in the Netherlands from 1998 – 2008.



E. coli in raw meat products of food-animals

Table 7.2 shows resistance percentages of *E. coli* strains isolated from raw meat products sampled at retail in the Netherlands by the Dutch Food and Consumer Product Safety Authority (VWA).

For most antibiotics, resistance percentages of *E. coli* strains isolated from poultry products were similar to those isolated as indicator organisms from faecal samples of Dutch broiler chickens (Table 7.1 and Table 7.2). However, as in previous years, resistance rates against the quinolones (nalidixic acid and ciprofloxacin) were lower in isolates from meat products than in isolates from faecal samples. Additionally, resistance against the aminoglycosides (gentamicin, kanamycin and streptomycin) as well as sulphamethoxazole was lower in meat products than in indicator bacteria from faecal samples.

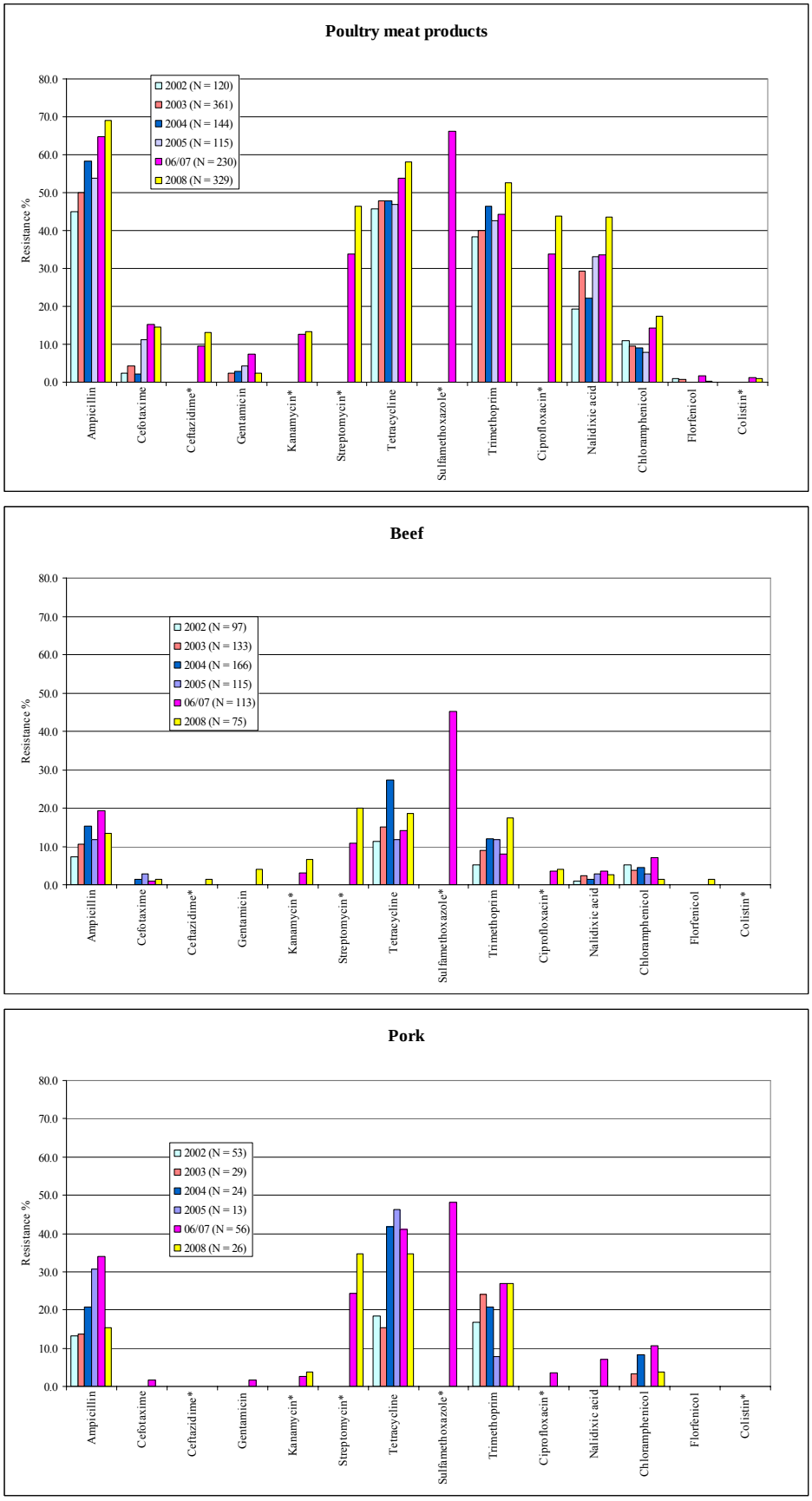
In beef samples, resistance rates were similar for both sources of isolates, except for a remarkable difference with regard to sulphamethoxazole (10.1% versus 90.7%). With regard to sulphamethoxazole, in all animal species levels of resistance were substantially higher in isolates from meat products compared to isolates from faecal samples. This may reflect a slight difference in methodology between the different labs as interpretation of test results can be difficult for sulphonamides.
Comparison of results from both pork and veal is complicated by the low number of isolates from meat products that are tested.

Table 7.2. Resistance (in %) of *E. coli* isolated from raw meat products at retail in the Netherlands in 2008.

	Poultry meat N = 329	Pork N = 26	Veal N = 13	Beef N = 75	Lamb N = 13
Ampicillin	69.0	15.4	61.5	13.3	23.1
Cefotaxime	14.6	0	0	1.3	0
Ceftazidime	13.1	0	0	1.3	0
Gentamicin	2.4	0	15.4	4.0	0
Kanamycin	13.4	3.8	46.2	6.7	15.4
Streptomycin	46.5	34.6	69.2	20.0	15.4
Tetracycline	58.1	34.6	92.3	18.7	23.1
Trimethoprim	53	26.9	76.9	17	7.7
Ciprofloxacin	43.8	0	15.4	4.0	7.7
Nalidixic acid	43.5	0	15.4	2.7	7.7
Chloramphenicol	17.3	3.8	15.4	1.3	15.4
Florfenicol	0.3	0	7.7	1.3	15.4
Colistin	0.9	0	0	0	0

Figure 7.3 shows trends in resistance in meat products from different animal species. Except for poultry meat, resistance profiles of *E. coli* isolated from meat in 2008 are similar to those in previous years. However, data from pork, veal and lamb have to be interpreted with care because of the low number of isolates.
Trends in resistance percentages from *E. coli* isolated from poultry meat show a tendency to increase, similar to resistance percentages from indicator bacteria isolated from faecal samples.

Figure 7.3. Trends in resistance (in%) of *E. coli* isolated from raw poultry meat products, beef, and pork, in the Netherlands from 2002 – 2008. Data from antibiotics marked with an asterix (*) are presented from 2007 onwards.



Enterococcus faecium and *Enterococcus faecalis*

This chapter presents information on resistance in *Enterococcus* species from food-producing animals in the Netherlands as indicator organisms for the occurrence and trends in resistance in Gram-positive bacteria. Isolates were selected from faecal samples of chickens, pigs, and cattle. In 2008, for 310 *Enterococcus faecalis* and 370 *E. faecium* strains MICs have been determined. In Table 7.3 MIC distributions are summarized for all *E. faecalis* and *E. faecium* strains isolated in 2008. Table 7.4 presents information on resistance rates in different animal species, specified for broiler chickens, slaughter pigs, veal calves and dairy cows.

Highlights
For both *E. faecalis* and *E. faecium*, high resistance levels were observed for tetracycline, erythromycin and streptomycin. Additionally, in *E. faecium* resistance rates are high for quinu/dalfopristin (73.8% vs. 1.9% in *E. faecalis*) and salinomycin (40% vs. 10.3% in *E. faecalis*). Ampicillin resistance was only observed in *E. faecium*. No resistance was observed against linezolid and florfenicol.
Compared to previous years, the number of high level ciprofloxacin resistant *E. faecalis* and *E. faecium* isolates (MIC ≥16 mg/l) in 2008 have increased in all considered farm animal species.
Vancomycin resistance was observed in *E. faecium* strains isolated from all animal species included in this survey, although at a very low level.

During 2008, changes in the test panels have been implemented. In the selection of antibiotics that are tested, in the course of 2008 florfenicol has been included and bacitracin and flavomycin have been removed. Additionally, concentration ranges of gentamicin and streptomycin have been modified. Previously, test ranges for gentamicin and streptomycin were specifically aimed at the detection of high level resistance against these antibiotics. The current test range permits the application of the epidemiological cut-off values as established by EUCAST.

For both *E. faecalis* and *E. faecium*, resistance was most frequently detected against tetracycline, erythromycin and streptomycin. Differences in resistance patterns among the two different enterococcal species were observed. In this survey, chloramphenicol resistance was higher in *E. faecalis*. Ampicillin resistance was solely observed in *E. faecium*, in all host animal species at relatively low levels. It appeared to be highest in isolates from poultry (15.7%). This is an increase compared to previous years in which resistance rates fluctuated at around 5% (Figure 7.4).

For streptomycin, the EUCAST epidemiological cut-off values demonstrate that 53.4% of the *E. faecalis* and 36.2% of the *E. faecium* strains are non wild-type, implicating the presence of acquired or mutational resistance mechanisms (Table 7.3).

With regard to gentamicin, using the EUCAST epidemiological cut-off value of 32 mg/l, 6.8% of the *E. faecalis* strains and 3.1% of the *E. faecium* strains were considered non-wild-type (Table 7.3).

Resistance against tetracycline was high in all animal species for both *E. faecalis* and *E. faecium*, with slightly lower levels of resistance in *E. faecium.* Trends over the years seem to be fairly stable in slaughter pigs and broiler chickens. In veal calves, tetracycline resistance shows a tendency to decrease in both *E. faecalis* and *E. faecium*. In contrast to this, levels in dairy cows seem to increase, although data have to be interpreted with care because of the limited number of strains tested.

Erythromycin resistance varies both with regard to host animal species as to enterococcal species tested. For *E. faecalis*, erythromycin resistance was highest in broiler chickens (81.5%) with slightly lower levels of resistance in the other animal species (55.6% in veal calves, 52.9% in dairy cows and 44% in pigs). In *E. faecium* isolated from broilers and calves, erythromycin resistance levels were equal (63.6% and 62.5% resp.), with slightly lower levels in strains from pigs (48.8%) and lowest levels in *E. faecium* from dairy cows (26.7%). This reflects the different usage patterns of macrolides in poultry, calves and pigs which is predominantly usage as flock or group treatment versus usage in dairy cows by injection only.

Also for bacitracin, resistance levels are slightly higher in *E. faecium* (36.5%) than in *E. faecalis* (24%). With regard to host species, highest levels were observed in broiler chickens and veal calves and slightly lower levels in pigs and dairy cows. However, trends over the years shows a clear decrease. This may be the consequence of the banning of bacitracin as a feed additive in 1999.

Table 7.3. MIC distributions (in %) for *Enterococcus faecalis* (N=310) and *E. faecium* (N=370) isolated in food animals in the Netherlands in 2008.

	MIC (%) distribution mg/L															
***E. faecalis* (n=310)**	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	R%
Ampicillin			95.8	3.2	1.0											0.0
Linezolid		0.3	15.5	80.6	3.5											0.0
Tetracyline		4.8	8.1	0.3			0.6	15.5	29.7	41.0						86.8
Erythromycin			14.5	6.1	7.7	0.6	1.6	1.6	0.3		67.4					71.6
Vancomycin		0.3	47.7	42.3	9.4	0.3										0.3
Ciprofloxacin		11.9	71.6	11.0	0.3		0.6	2.9	1.6							5.2
Bacitracin								5.3	46.2	24.4	2.7	21.3				24.0
Flavomycin					98.2	1.8										0
Salinomycin		3.9	39.4	7.7	38.7	10.0	0.3									10.3
Quinu/dalfopristin		0.3	0.3	0.6	1.3	31.0	59.0	5.5	1.9							1.9
Gentamicin*						22.7	64.8	5.7		1.1	5.7					6.8
Streptomycin*							1.1		4.5	38.6	2.3			53.4		53.4
Chloramphenicol			1.0	0.3	1.9	76.8	9.4	0.6	8.1	1.9						10.0
Florfenicol*				4.7	95.3											0.0

	MIC (%) distribution mg/L															
***E. faecium* (n=370)**	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	R%
Ampicillin			46.5	21.4	19.7	8.4	0.8	0.3	1.6	1.4						12.4
Linezolid			3.5	62.4	34.1											0.0
Tetracyline		26.8	5.7			0.3	0.8	2.2	16.5	47.8						67.6
Erythromycin			19.5	16.5	8.4	2.2	0.8	0.3	0.5	0.3	51.6					55.7
Vancomycin		53.2	38.4	5.9	1.6	0.3				0.5						0.8
Ciprofloxacin		3.0	19.7	28.9	34.1	11.4	2.7	0.3								14.3
Bacitracin				1.2	9.0	4.5			17.2	31.6	7.8	28.7				36.5
Flavomycin										1.2	2.9	12.3	83.6			100.0
Salinomycin		1.1	18.6	29.2	11.1	39.2	0.5		0.3							40.0
Quinu/dalfopristin		11.9	14.3	18.9	43.2	10.5	1.1									73.8
Gentamicin*					7.9	44.9	37.8	6.3			0.8		2.4			3.1
Streptomycin*							1.6	2.4	38.6	21.3	0.8	1.6	1.6	32.3		36.2
Chloramphenicol			0.3		4.9	67.8	14.6	11.6	0.8							0.8
Florfenicol*				7.1	92.9											0.0

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

*During the course of 2008, MIC test ranges for gentamicin, streptomycin and florfenicol have been modified. MIC distributions for gentamicin, streptomycin and in this table represent the new range. Data for gentamicin, streptomycin, and florfenicol represent test results of 85 E. faecalis and 126 E. faecium strains. MICs for bacitracin and flavomycin have been determined for 225 E. faecalis and 244 E. faecium strains.

In both enterococcal species, decreased vancomycine susceptibility was identified. An *E. faecalis* strain with an MIC of 8 mg/L was recovered from a pig sample. An analysis on the genetic basis of resistance in this isolate was not performed. Among the *E. faecium* strains, three isolates with decreased vancomycine susceptibility were isolated, from a variety of host species. These included calves (one isolate with an MIC of 8 mg/l), chickens (one isolate, MIC of 128 mg/l) and pigs (one isolate with an MIC of 128mg/l). These data indicate that, although at a relatively low rate, vancomycine resistance determinants (*van*A) are still present in all main food animal sectors in the Netherlands.

Linezolid (first approved for use in humans in 2000) is indicated for the treatment of severe infections by Gram positive bacteria that are resistant to other antibiotics and is reserved for potentially untreatable infections. Acquired resistance has been reported in hospital settings, but has remained stable and extremely low. None of the *E. faecalis* and *E. faecalis* strains isolated in this study were resistant against linezolid, as might be expected since no related compound has been used in animals.

Table 7.4. Resistance percentages (%) of *E. faecalis* and *E. faecium* isolated from faeces from slaughter pigs, broilers, veal calves, and dairy cows in the Netherlands in 2008.

	% resistance (N strains tested)			
E. faecalis	**Slaughter pigs**	**Broiler chickens**	**Veal calves**	**Dairy cows**
Ampicillin	0 (50)	0 (216)	0 (27)	0 (17)
Linezolid	0 (50)	0 (216)	0 (27)	0 (17)
Tetracyline	88.0 (50)	88.0 (216)	74.1 (27)	88.2 (17)
Erythromycin	44.0 (50)	81.5 (216)	55.6 (27)	52.9 (17)
Vancomycin	2.0 (50)	0 (216)	0 (27)	0 (17)
Ciprofloxacin	6.0 (50)	4.6 (216)	7.4 (27)	5.9 (17)
Bacitracin	17.8 (45)	25.7 (148)	30 (20)	16.7 (12)
Flavomycin	0 (45)	0 (148)	0 (20)	0 (12)
Salinomycin	0 (50)	13.9 (216)	7.4 (27)	0 (17)
Quinu/dalfopristin	2.0 (50)	0.5 (216)	7.4 (27)	11.8 (17)
Gentamicin	0.0 (5)	0.0 (68)	0.0 (7)	0.0 (5)
Streptomycin	60.0 (5)	57.4 (68)	42.9 (7)	40.0 (5)
Chloramphenicol	4.0 (50)	8.8 (216)	25.9 (27)	17.6 (17)
Florfenicol	0 (5)	0 (68)	0 (7)	0 (5)
E. faecium	**Slaughter pigs**	**Broiler chickens**	**Veal calves**	**Dairy cows**
Ampicillin	10 (80)	15.7 (197)	4.2 (48)	11.1 (45)
Linezolid	0 (80)	0 (197)	0 (48)	0 (45)
Tetracyline	85.0 (80)	70.6 (197)	60.4 (48)	31.1 (45)
Erythromycin	48.8 (80)	63.5 (197)	62.5 (48)	26.7 (45)
Vancomycin	1.3 (80)	0.5 (197)	2.1 (48)	0 (45)
Ciprofloxacin	11.3 (80)	17.3 (197)	12.5 (48)	8.9 (45)
Bacitracin	22.6 (62)	46.5 (114)	42.1 (38)	20.0 (30)
Flavomycin	100 (62)	100 (114)	100 (38)	100 (30)
Salinomycin	35 (80)	55.3 (197)	6.3 (48)	17.8 (45)
Quinu/dalfopristin	88.8 (80)	71.1 (197)	58.3 (48)	75.6 (45)
Gentamicin	0.0 (18)	2.4 (83)	10.0 (10)	0.0 (15)
Streptomycin	33.3 (18)	32.5 (83)	50.0 (10)	20.0 (15)
Chloramphenicol	0 (80)	0.5 (197)	4.2 (48)	0 (45)
Florfenicol	0 (18)	0 (83)	0 (10)	0 (15)

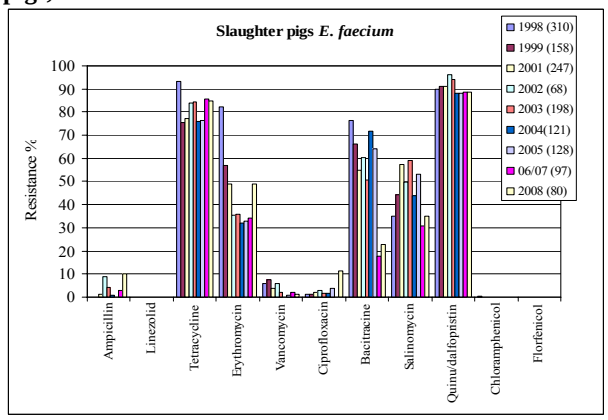
The streptogramin combination of quinupristin and dalfopristin (synercid®) is a last resort drug for treatment of infections by staphylococci and vancomycin-resistant *Enterococcus* faecium (VRE). Acquired resistance against the quinupristin/dalfopristin combination was observed in *E faecium*, in 73.8% of the isolates, although the cut-off values used seem somewhat arbitrary clinical resistance, as defined by an MIC >4 mg/l was identified in 11.6% of the *E. faecium* strains in this survey.

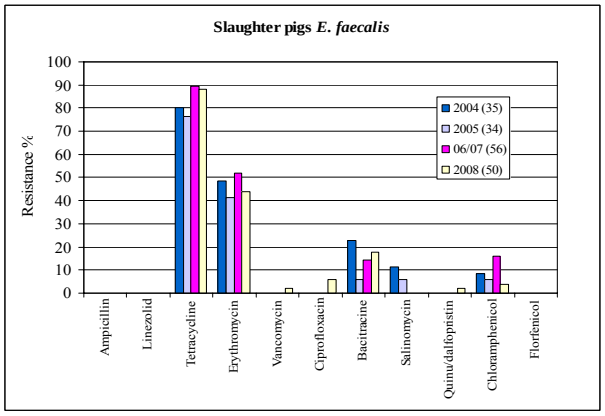
Acquired ciprofloxacin resistance shows some variation among the animal species, and appears to slightly increase over the years. Overall, resistance levels are somewhat higher in *E. faecium* compared to *E. faecalis*.
As in previous years, in 2008 high level ciprofloxacin resistant *E. faecalis* and *E. faecium* isolates were observed (MIC ≥16 mg/l) in increasing numbers. Rates of high level resistance in *E. faecalis* were slightly lower compared to those in *E. faecium* as shown in Table 7.3. The majority of the strains were isolated from broiler chickens, reflecting the extent of the quinolone use in this sector. However, high level resistant *E. faecalis* and *E. faecium* strains were also detected in samples taken from pigs, veal calves and dairy cows.

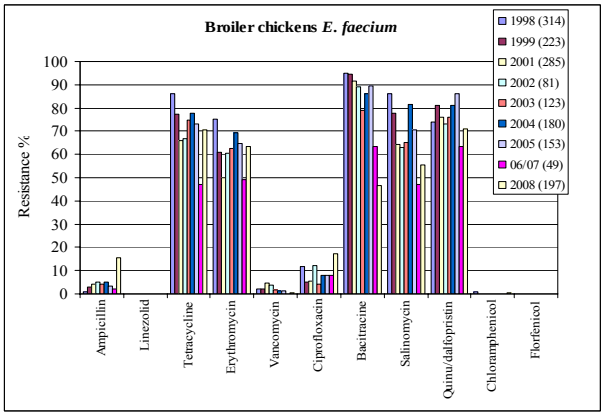
Chloramphenicol resistance was low in all animal species with the exception of *E. faecalis* isolates from veal calves and dairy cows. Although the number of tested strains was limited, 25.9% and 17.6% resp. showed decreased susceptibility. No florfenicol resistance was observed.

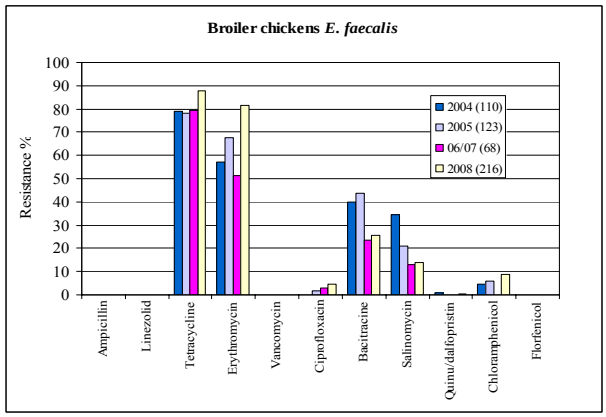
Acquired salinomycin resistance was highest in *E. faecalis* and *E. faecium* strains from broilers, which can be explained by the use of ionophores as coccidiostatic agents in the feed of these animals.

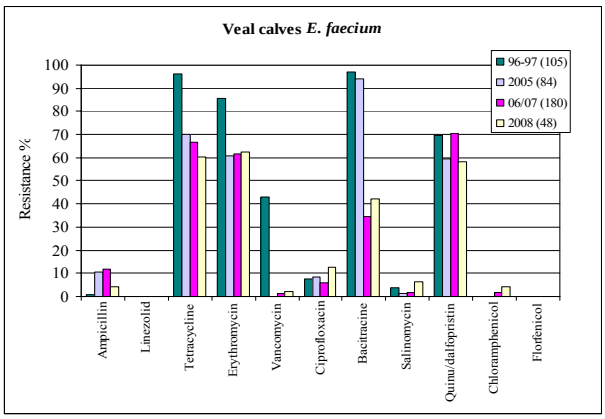
Figure 7.4. Trends in resistance percentages of *Enterococcus faecium* and *E. faecalis* isolated from slaughter pigs, broilers and veal calves in the Netherlands from 1996 – 2008.

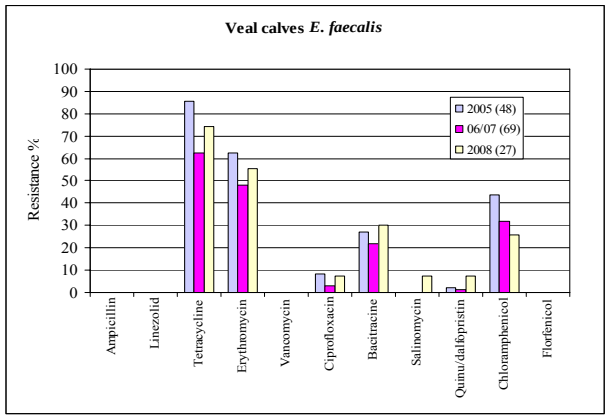


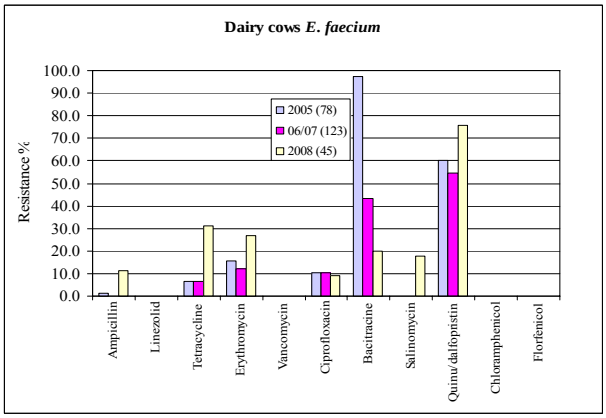


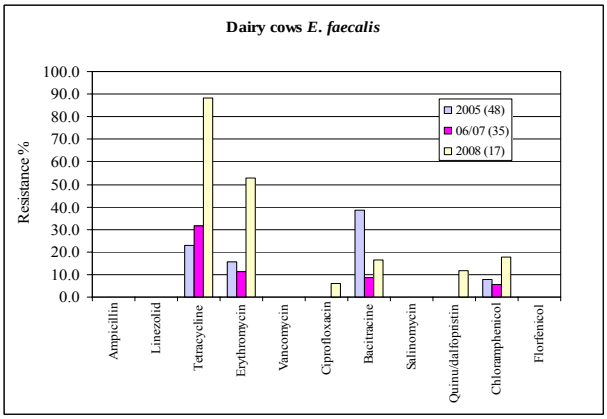












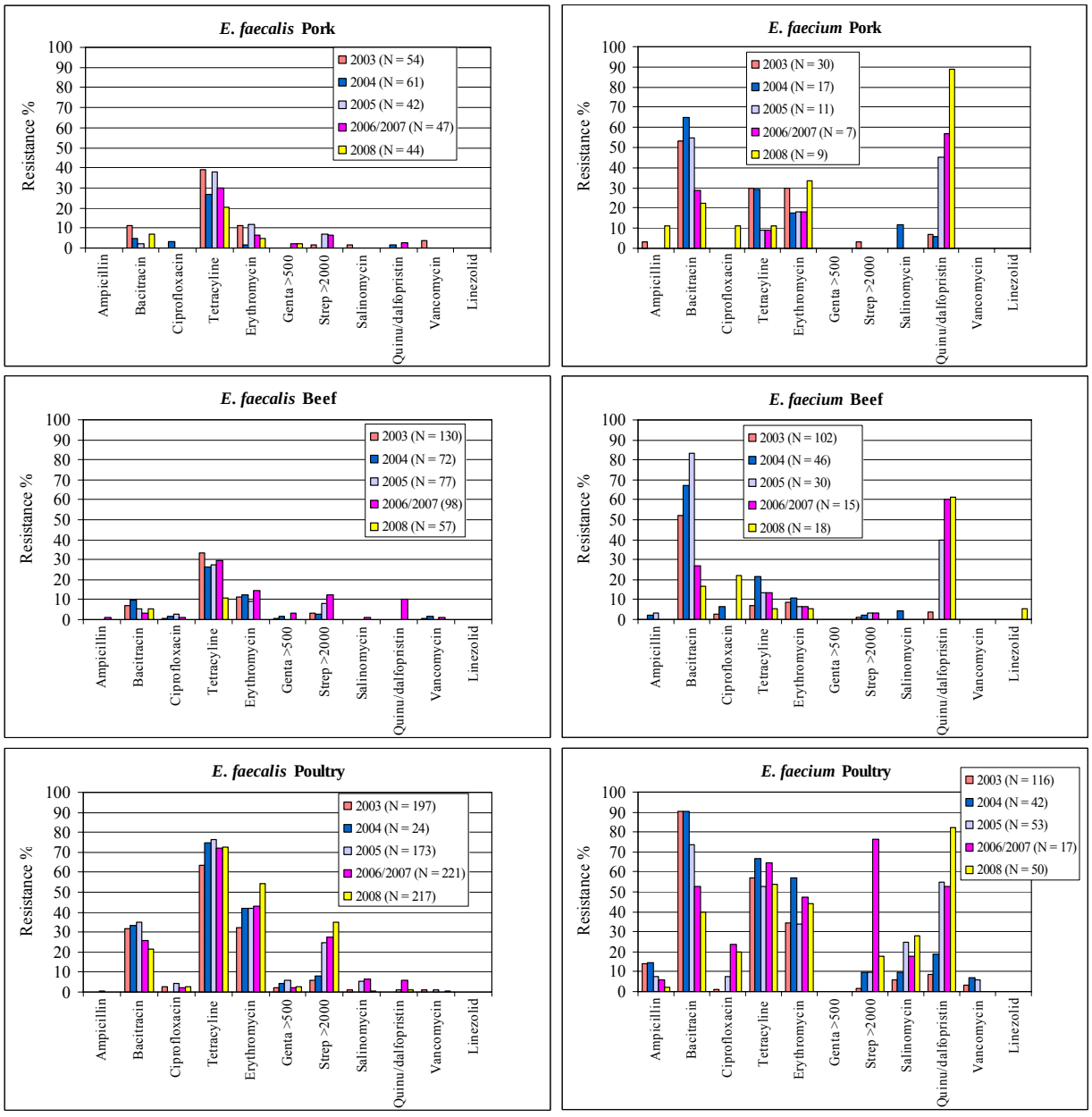
***Enterococcus faecalis* and *E. faecium* in raw meat products of food-animals**

Table 7.5. Resistance % of *E. faecalis* and *E. faecium* strains isolated from raw meat products from poultry, beef, pork, lamb and veal in the Netherlands in 2008.

E. faecalis	**Pork (N = 44)**	**Poultry (N = 217)**	**Veal (N = 11)**	**Beef (N = 57)**	**Lamb (N = 15)**
Ampicillin	0	0	0	0	0
Linezolid	0	0	0	0	0
Tetracyline	20.5	72.8	36.4	10.5	73.3
Erythromycin	4.5	54.4	18.2	0	20.0
Vancomycin	0	0.5	0	0	0
Ciprofloxacin	0	2.8	0	0	0
Bacitracin	6.8	21.7	0	5.3	6.7
Salinomycin	0	0.5	0	0	0
Quinu/dalfopristin	0	0.9	0	0	0
Genta >500	2.3	2.8	0	0	0
Strep >2000	0	35.0	18.2	0	20.0
Chloramphenicol	2.3	7.8	0	0	0
E. faecium	**Pork (N = 9)**	**Poultry (N = 50)**	**Veal (N = 4)**	**Beef (N = 18)**	**Lamb (N = 2)**
Ampicillin	11.1	2.0	0	0	0
Linezolid	0	0	0	5.6	0
Tetracyline	11.1	54.0	25.0	5.6	0
Erythromycin	33.3	44.0	25.0	5.6	0
Vancomycin	0	0	0	0	0
Ciprofloxacin	11.1	20.0	0	22.2	0
Bacitracin	22.2	40.0	25.0	16.7	50.0
Salinomycin	0	28.0	0	0	0
Quinu/dalfopristin	88.9	82.0	75.0	61.1	0
Genta >500	0	0	0	0	0
Strep >2000	0	18.0	0	0	0
Chloramphenicol	0	0	0	0	0

Figure 7.5 illustrates resistance rates over the years for both *E. faecalis* and *E. faecalis*, specified for the various food animal species.
When resistance rates in *E. faecalis* and *E. faecium* isolated from food products are compared to those from faecal samples, levels are either comparable (as for ciprofloxacin, quinu/dalfopristin and chloramphenicol) or lower (as for ampicillin, tetracycline, erythromycin, bacitracin and salinomycin.

Figure 7.5. Trends in resistance percentages in E. faecalis and E. faecium isolated from raw meat products from poultry, beef and pork in the Netherlands from 2003 to 2008.



8. Animal pathogens

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

In collaboration with the Animal Health Service in Deventer, the Netherlands, strains isolated from diagnostic specimens are tested for susceptibility by broth microdilution. Isolates originated from diagnostic samples either obtained at autopsy from cattle suffering from respiratory diseases (the majority) or from live animals by broncheo-alveolar lavage. Data are available from 1996 onwards. For some years, the number of strains isolated per year is limited. Therefore resistance data on respiratory disease pathogens from cattle have been reported every two to three years.

Highlights
In general, resistance levels were low in both *Pasteurella multocida* and *Mannheimia haemolytica* strains from cattle with respiratory disease. With the exception of tetracycline resistance in *Mannheimia*, resistance levels were all below 10%, which is regarded as the threshold for the empirical use of antibiotics.
For most antibiotics tested, resistance levels in *Mannheimia haemolytica* are higher than in *Pasteurella multocida*.

In Table 8.1 the MIC distributions are presented for both *Pasteurella multocida* and *Mannheimia haemolytica*, isolated in 2008. In Figure 8.1 the trends in resistance percentages are presented for both pathogens from 1996 to 2008.

In previous chapters epidemiological cut off values for the wild type distribution have been used. Because of a lack of epidemiological cut off values for most antibiotics that were tested, clinical breakpoints were used for the calculation of resistance percentages for animal pathogens. For three antibiotics EUCAST has defined epidemiological cut-off values for *Pasteurella multocida* and *Mannheimia haemolytica*. For gentamicin, the epidemiological cut-off value and the clinical breakpoint are equal (>4 mg/L). For ampicillin, the epidemiological cut off value for both microorganisms (0.5 mg/L) is lower than the clinical breakpoint (16 mg/L), however the percentage resistant (or decrease susceptible) strains are similar. Also for tetracycline, the epidemiological cut-off values (>4 mg/L for *Pasteurella multocida* and >2 mg/L for *Mannheimia haemolytica*) are lower than the clinical breakpoints (>8 mg/L for both microorganisms), and this leads to minor (*Pasteurella multocida*) or no (*Mannheimia haemolytica*) changes in resistance percentages.

For most antibiotics the resistance levels in *Mannheimia haemolytica* were higher compared to *Pasteurella multocida*. The most striking difference was tetracycline, for which more than half of the *Mannheimia haemolytica* isolates were resistant in contrast to only 7.7% of the *Pasteurella multocida* isolates. For a number of antimicrobial agents resistance was only observed in *Mannheimia haemolytica* and not in *Pasteurella multocida* (amoxicillin, flumequine, tilmicosin and tulathromycin). On the other hand resistance against florfenicol and spectinomycin was only detected in *Pasteurella multocida* (3.8% and 7.7% respectively). In both pathogens, no resistance was observed against ceftiofur and the combination of trimethoprim with sulphamethoxazole. Enrofloxacin en flumequine MICs show a typical bi-modal distribution pattern for *Mannheimia haemolytica*, with clearly reduced susceptible populations with MICs ≥0.125 mg/L for enrofloxacin and 2–8 mg/L for flumequine. The current clinical breakpoint used in the Netherlands for flumequine cuts through this reduced susceptible population and should be redefined to >1 mg/L for this bacterial species.

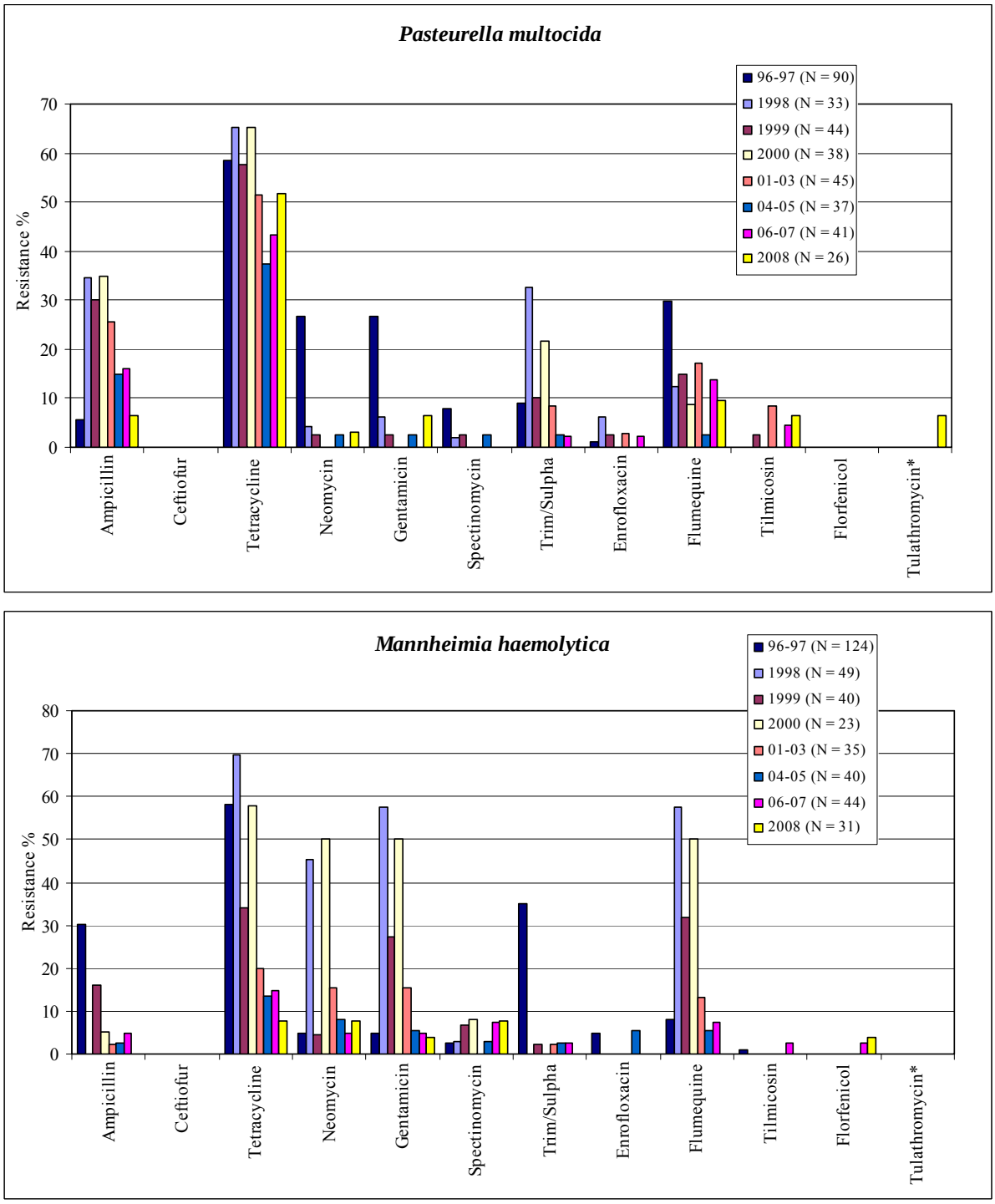
Table 8.1. MIC-distributions (in %) for bovine respiratory disease pathogens *Pasteurella multocida* and *Mannheimia haemolytica* isolate from Dutch cattle by the Animal Health Service in Deventer in 2008.

	MIC % distribution (µg/ml)																
P. multocida (26)	**0.015**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**R%**
Ampicillin					96.2	3.8											0
Ceftiofur			96.2	3.8													0
Tetracycline						30.8	30.8	15.4	11.5	3.8	3.8		3.8				7.7
Neomycin							11.5	38.5	26.9	15.4		3.8		3.8			7.7
Gentamicin						7.7	26.9	53.8	7.7					3.8			3.8
Spectinomycin										7.7	57.7	26.9				7.7	7.7
Trim/sulpha				80.8	11.5	3.8	3.8										0
Enrofloxacin		96.2	3.8														0
Flumequine					88.5		3.8	3.8	3.8								0
Tilmicosin						3.8	7.7	50.0	23.1	11.5	3.8						0
Tulathromycin						3.8	34.6	46.2	15.4								0
Florfenicol					7.7	84.6	3.8				3.8						3.8
M. haemolytica (31)	**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**	**512**	**R%**
Ampicillin					90.3	3.2							6.5				6.5
Ceftiofur			96.8	3.2													0
Tetracycline						3.2	38.7	6.5			19.4	25.8	6.5				51.6
Neomycin								6.5	51.6	38.7		3.2					3.2
Gentamicin							6.5	87.1			3.2		3.2				6.5
Spectinomycin											12.9	87.1					0
Trim/sulpha				64.5	16.1		16.1	3.2									0
Enrofloxacin		51.6	6.5	3.2	9.7	29.0											0
Flumequine					58.1			3.2	29.0	9.7							9.7
Tilmicosin						3.2			38.7	45.2	6.5		3.2	3.2			6.5
Tulathromycin							3.2	6.5	71.0	9.7	3.2		3.2	3.2			6.5
Florfenicol						9.7	87.1	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. The vertical bars indicate the clinical breakpoints.

Figure 8.1 shows the resistance percentages over the years. Resistance against ampicillin seems to show a decreasing trend in both pathogens. However, percentages of most antibiotics vary substantially over the years because of the limited number of available isolates. Trend analysis is further complicated by a potential bias in the population of isolates. The resistance data are mostly based on post mortem samples and probably reflect a worst-case scenario of resistance in these pathogens. Still, they are a valuable source of information regarding the occurrence and prevalence of resistance determinants.

Figure 8.1. Trends in resistance (in %) for *P. multocida* and *M. haemolytica* isolated from 1996 – 2008 in the Netherlands.



*Tulathromycin has been tested since 2007.

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

Highlights
Resistance levels in *E. coli* strains isolated from milk samples from dairy cows were generally low to moderate. Highest levels were observed against tetracycline, streptomycin and ampicillin. As in previous years, ESBL producing *E. coli* isolates were isolated from milk samples from dairy cows. In comparison, the coliform bacteria showed a higher level of resistance against ampicillin (85%) and amoxicillin-clavulanic acid (22%).
Staphylococcus aureus strains had low levels of resistance against most antibiotics. Again, a methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from a milk sample from a cow with mastitis.
In general, coagulase-negative staphylococci were more resistant. In 2008, 53% was penicillin resistant and 3% oxacillin resistant (*mecA* positive). Based on epidemiological cut off values, 24% of the coagulase-negative staphylococci had reduced susceptibility with regard to clindamycin, compared to 1% of the *S. aureus* isolates.

In Table 8.2, the MIC distributions are presented for both *E. coli* and coliform bacteria (mostly *Klebsiella* and *Enterobacter* species), isolated from milk samples from cows with (sub)clinical mastitis in 2008. In Figure 8.2 the trends in resistance percentages are presented for *E. coli* and coliform bacteria from 2002 to 2008.

E. coli strains isolated from milk samples from cows with intramammary infections generally showed low to moderate resistance levels. Highest levels were observed against tetracycline (16.2%), streptomycin (13.1%) and ampicillin (11.1%). In coliform bacteria, resistance levels against the first two mentioned antibiotics were similar (17% for tetracycline and 14% for streptomycin). However, resistance levels in coliform bacteria against ampicillin was much higher (85%), while 22% of the coliform bacterial strains were also resistant against the combination of amoxicillin with clavulanic acid. This is due to the commonly present beta-lactamases in *Klebsiella* and *Enterobacter* spp.

Both in *E. coli* and coliform strains resistance against second (cefuroxime) and third (cefoperazone) generation of cephalosporins was detected. Levels were relatively low when clinical breakpoints were applied. However, using epidemiological cut-off values (8 mg/L for cefuroxime and 1 mg/L for cefoperazone), which is a more sensitive measure for obtained resistance determinants, 4% of the *E. coli* strains can be regarded as non - wild type with regard to cefuroxime while 8% show reduced susceptibility for cefoperazone.
Another worrisome finding is the resistance against fourth generation cephalosporins (cefquinome) in *E. coli* isolates from mastitis samples, which is also indicative for the presence of Extended Spectrum Beta-Lactamase (ESBL). This may have serious implications for the treatment outcome, as bacteria producing ESBL have to be considered resistant against all beta-lactam antibiotics (including all cephalosporins). This is the single most important class of antimicrobials for the treatment (and prevention) of mastitis in cattle.

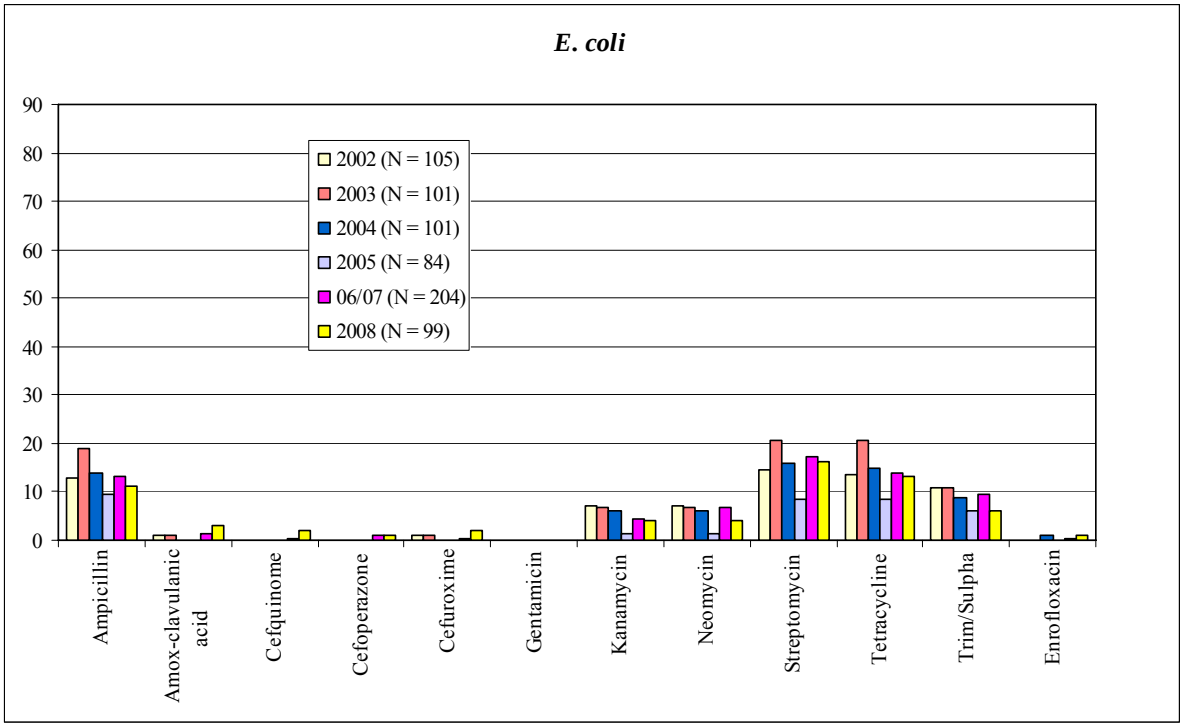
Figure 8.2 showed that since 2002 the resistance levels in both *E. coli* and in coliform bacteria from (sub)clinical mastitis the resistance levels remain stable except resistance against 3rd and 4th generation cephalosporins in *E.* coli which was first detected in 2006/2007.

Table 8.2. 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 2008.

E. coli	**MIC (%) distribution mg/L**													
(99)	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**R%**
Ampicillin						3.0	38.4	38.4	9.1			11.1		11.1
Amox-clavulanic acid						1.0	19.2	51.5	23.2	2.0	1.0	1.0	1.0	3.0
Cefquinome		79.8	13.1	1.0	3.0	1.0			1.0	1.0				2.0
Cefoperazone				75.8	15.2	1.0	3.0	3.0	1.0			1.0		1.0
Cefuroxime							7.1	62.6	26.3	2.0	2.0			2.0
Tetracycline						3.0	26.3	54.5				16.2		16.2
Gentamicin					53.5	43.4	3.0							0.0
Kanamycin							2.0	70.7	23.2				4.0	4.0
Neomycin						35.4	56.6	4.0			4.0			4.0
Streptomycin							1.0	19.2	54.5	11.1	1.0	5.1	8.1	13.1
Enrofloxacin	58.6	40.4							1.0					1.0
Trim/Sulpha			91.8	1.0	1.0						6.1			6.1
Coliform	**MIC (%) distribution mg/L**													
(100)	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**R%**
Ampicillin						3.0	1.0	2.0	2.0	7.0	33.0	52.0		85.0
Amox-clavulanic acid						2.0	50.0	16.0	7.0	3.0	5.0	11.0	6.0	22.0
Cefquinome		72.0	19.0	7.0	2.0									0.0
Cefoperazone				60.0	11.0	8.0	12.0	8.0				1.0		1.0
Cefuroxime						5.0	39.0	30.0	13.0	7.0	6.0			6.0
Tetracycline						12.0	52.0	17.0	2.0		1.0	16.0		17.0
Gentamicin				8.0	87.0	5.0								0.0
Kanamycin						7.0	46.0	29.0	6.0	2.0	2.0	1.0	7.0	8.0
Neomycin					19.0	71.0	4.0	1.0		1.0	4.0			4.0
Streptomycin							20.0	56.0	2.0	4.0	4.0	3.0	11.0	14.0
Enrofloxacin	33.0	57.0	8.0	2.0										0.0
Trim/Sulpha			85.0	5.0	5.0	4.0					1.0			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 clinical breakpoints.

Figure 8.2. Trends in resistance percentages for *E. coli* and coliform bacteria isolated milk samples of cattle with intramammary infections in the Netherlands from 2002 – 2008.



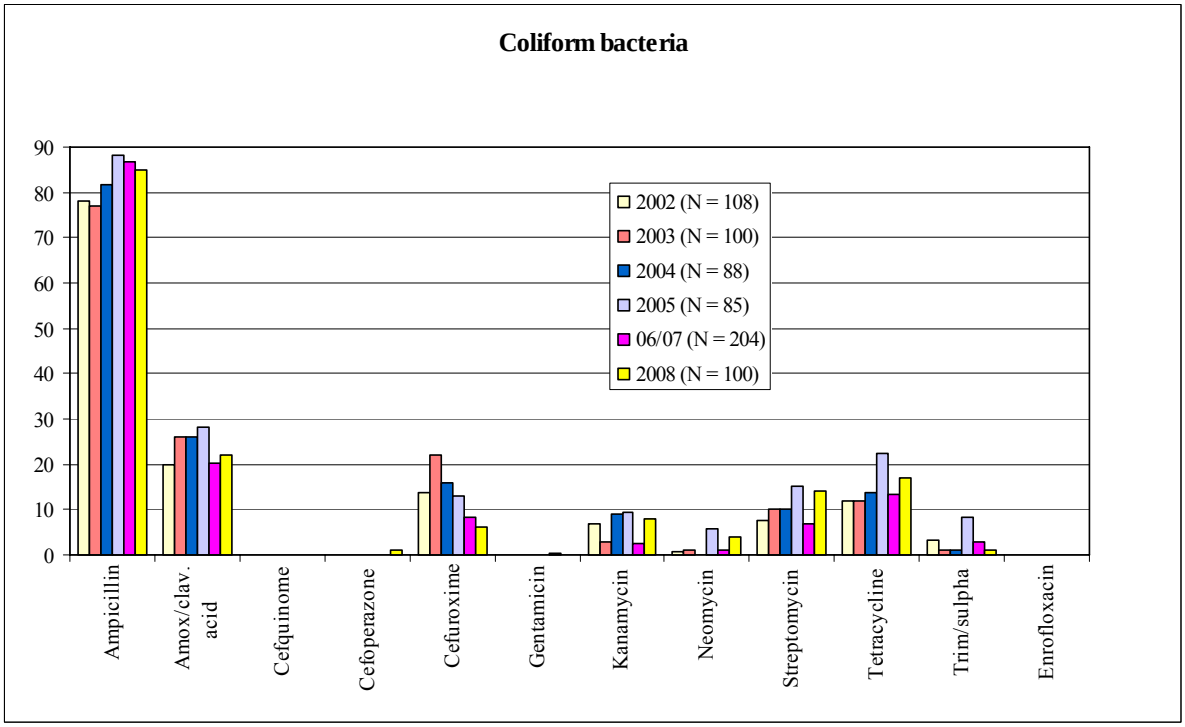


Table 8.3 presents the MIC distributions for *Staphylococcus aureus* and coagulase-negative *Staphylococcus* species (CNS), isolated from milk samples from cows with (sub)clinical mastitis in 2008. In Figure 8.3 the trends in resistance percentages are presented from 2002 to 2008.

In the Netherlands, resistance levels in *Staphylococcus* strains from mastitis samples have been historically low, with highest levels observed against penicillin. Penicillin resistance based on MICs and cefinase tests show similar results compared to previous years (9.9% of the *S. aureus* and 53% of the CNS were resistant).
In 2008, again an oxacillin resistant *S. aureus* was isolated from a milk sample which was subsequently confirmed as methicillin-resistant *S. aureus* (MRSA) by the presence of the *mecA*-gene, and 442 bp *S. aureus* specific gene fragment according to Martineau et al. (1998)[7]. Also in three oxacillin resistant CNS the *mecA*-gene was identified. As a result, all oxacillin-resistant strains were also classified as resistant against amoxicillin-clavulanic acid and cephalothin.
Based on clinical breakpoints, cephalothin resistance in a *S. aureus* isolate would have been missed. However, using the EUCAST epidemiological cut-off value for cephalotin (1 mg/L) the strain would have been identified as non-wild type, indicating the presence of resistance determinants. This illustrates the usefulness of this kind of cut-off values.

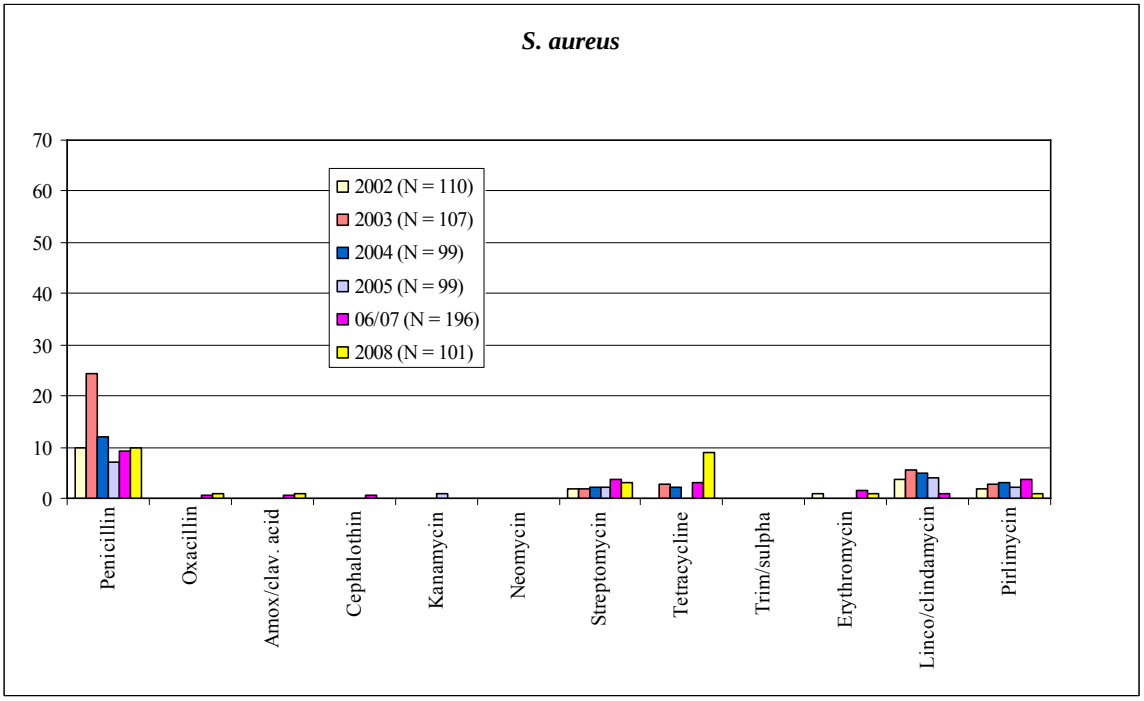
Table 8.3. MIC-distributions (in %) of *S. aureus* and coagulase-negative staphylococci (CNS) isolated from (sub)clinical mastitis cases in dairy cattle by the Animal Health Service in Deventer in 2008.

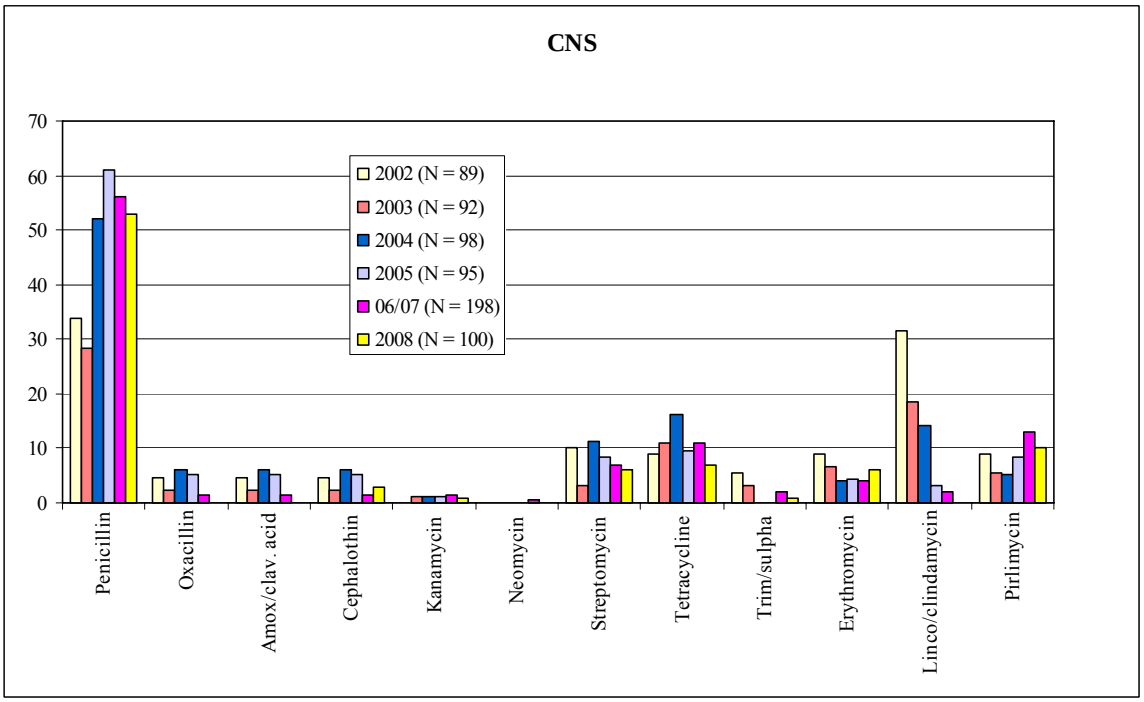
S. aureus	**MIC (%) distribution mg/L**												
(101)	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**R%**
Penicillin	84.2	5.9	1.0				2.0	4.0	3.0				9.9
Oxacillin		5.9	42.6	41.6	7.9	1.0			1.0				1.0#
Amox-clavulanic acid		13.9	65.3	10.9	6.9	2.0		1.0					1.0#
Cephalothin		24.8	64.4	9.9		1.0							1.0#
Kanamycin					2.0	18.8	62.4	16.8					0
Neomycin			5.0	34.7	58.4	2.0							0
Streptomycin						3.0	16.8	67.3	9.9		2.0	1.0	3.0
Tetracycline			2.0	65.3	23.8					8.9			8.9
Trim/sulpha		98.0	1.0	1.0									0
Erythromycin			70.3	28.7						1.0			1.0
Clindamycin		76.2	22.8	1.0									1.0^
Pirlimycin			5.9	48.5	44.6		1.0						1.0
CNS	**MIC (%) distribution mg/L**												
(100)	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**R%**
Penicillin	51.0	14.0	10.0	4.0	5.0	4.0	2.0	1.0	9.0				53.0*
Oxacillin	3.0	18.0	28.0	35.0	13.0			1.0	2.0				3.0#
Amox-clavulanic acid		27.0	47.0	20.0	4.0	2.0							3.0#
Cephalothin	1.0	27.0	43.0	21.0	6.0	2.0							3.0#
Kanamycin				10.0	32.0	38.0	16.0	2.0	1.0			1.0	1.0
Neomycin		54.0	19.0	12.0	13.0	1.0		1.0					0
Streptomycin					15.0	31.0	21.0	25.0	2.0		3.0	3.0	6.0
Tetracycline		1.0	18.0	55.0	14.0	4.0	1.0			7.0			7.0
Trim/sulpha		71.0	23.0	5.0				1.0					1.0
Erythromycin		6.0	47.0	34.0	6.0	0.0	1.0	1.0	3.0	2.0			6.0
Clindamycin	12.0	35.0	29.0	17.0	6.0	1.0							0
Pirlimycin		2.0	21.0	41.0	19.0	7.0	6.0	4.0					10.0

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 clinical breakpoints.
* 18 CNS isolates (18%) with penicillin MIC ≤0.06 mg/L positive for penicillinase production based on the cefinase test were classified resistant against penicillin. Therefore the accurate penicillin resistance percentage was 53.0%
# All S. aureus isolates with MIC >2 µg/ml and CNS isolates with MIC >4 µg/ml against oxacillin were *mec*A-positive. These isolates were also classified R for amoxicillin-clavulanic acid and cephalothin.
^ Inducible macrolides-lincosamides-streptogramin B resistance was detected in a single *S. aureus* isolate, based on which it was also classified as resistant against clindamycin.

[7] Martineau, F., et al., *Species-specific and ubiquitous-DNA-based assays for rapid identification of Staphylococcus aureus.* J Clin Microbiol, 1998. **36**(3): p. 618-23.

Figure 8.3. Trends in resistance percentages for *S. aureus* and coagulase-negative staphylococci (CNS) isolated from milk samples of cattle with intramammary infections in the Netherlands from 2002 - 2008.





Resistance against the aminoglycosides kanamycin, neomycin and streptomycin was rare for both *S. aureus* and CNS, with highest levels for streptomycin (6% for CNS and 3% for *S. aureus*).

Resistance against the related compounds clindamycin and pirlimycin was low in *S. aureus* (1% for both agents). Pirlimycin resistance was 10% in CNS, while no resistance was observed against clindamycin.
Trends over the years appear to be fairly stable for most antibiotics. The apparent decrease in resistance against the lincosamides may be affected by a switch in testing clindamycin instead of lincomycin from 2004 onwards. Since 2006, MRSA isolates are incidentally detected in mastitis in dairy cattle, indicating a low prevalence in these animals.

Table 8.4 presents the MIC distributions for *Streptococcus* (*S.*) *uberis* and *S. dysgalactiae*, isolated from milk samples from cows with intramammary infections in 2008. In *S. uberis* and *S. dysgalactiae* no resistance was observed against the beta-lactam antibiotics (penicillin and cephalothin). However, for both antibiotics the MIC-distribution is clearly bi-modal, indicating the presence of an acquired resistance mechanism. No resistance was observed for trimethoprim with sulphamethoxazole as well.
High levels of resistance were observed for tetracycline (63.6% of the *S. dysgalactiae* and 45.9% of the *S. uberis* strains). Also resistance against the lincosamide antibiotics occurred frequently, with highest levels in *S. uberis*. For these antibiotics the MIC-distribution is multi-modal and the clinical breakpoints used seem to cut through a reduced susceptible population. The result is that the resistance percentages that are based on the breakpoints used are an underestimation of the true resistant population. Resistance against erythromycin was most common in *S. uberis.*

Table 8.4. MIC-distributions (in %) of *S. uberis* and *S. dysgalactiae* isolated from mastitis milk samples from Dutch cattle by the Animal Health Service in 2008.

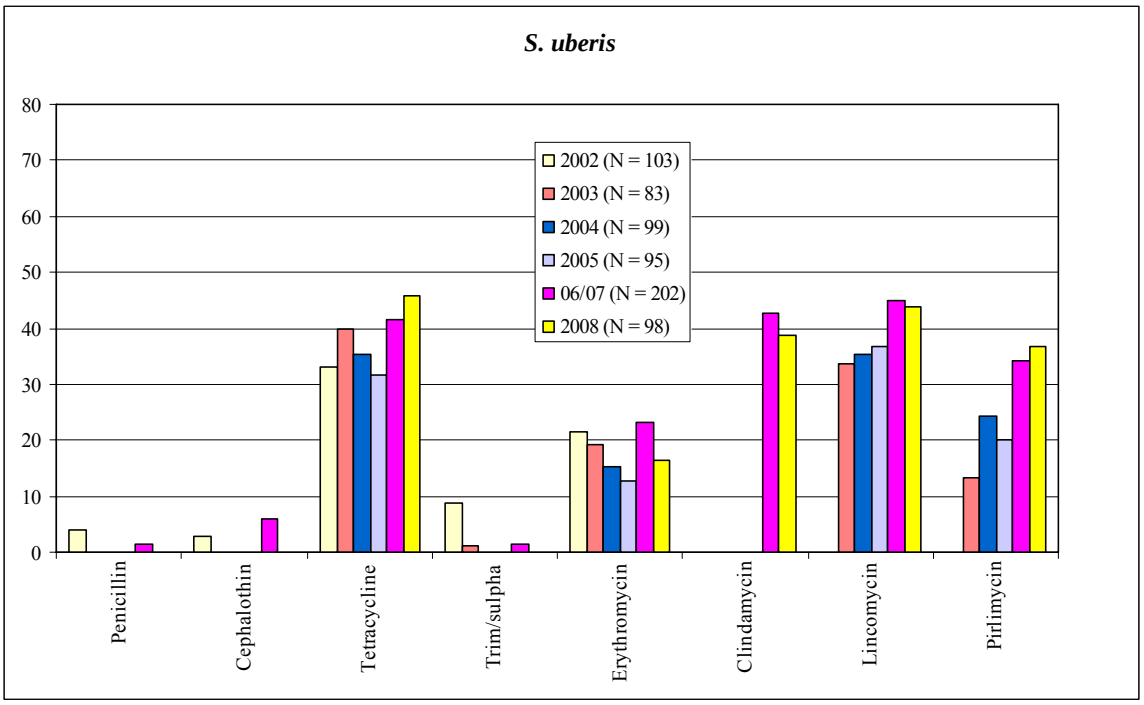
S. uberis	**MIC % distribution (µg/ml)**															
(98)	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Penicillin	21.4	12.2	6.1	6.1	45.9	8.2										0
Cephalothin				21.4	13.3	14.3	46.9	4.1								0
Erythromycin		3.1	73.5	5.1	2.0			2.0					14.3			16.3
Clindamycin		6.1	36.7	12.2	3.1	1.0		2.0	20.4	5.1		1.0	12.2			38.8
Lincomycin				27.6	5.1		2.0	9.2	12.2	3.1			40.8			43.9
Pirlimycin			27.6	27.6	2.0	2.0	1.0	3.1	16.3	7.1		1.0	12.2			36.7
Trim/sulpha		6.1	34.7	55.1	4.1											0
Tetracycline					7.1	44.9	2.0					4.1	29.6	11.2	1.0	45.9

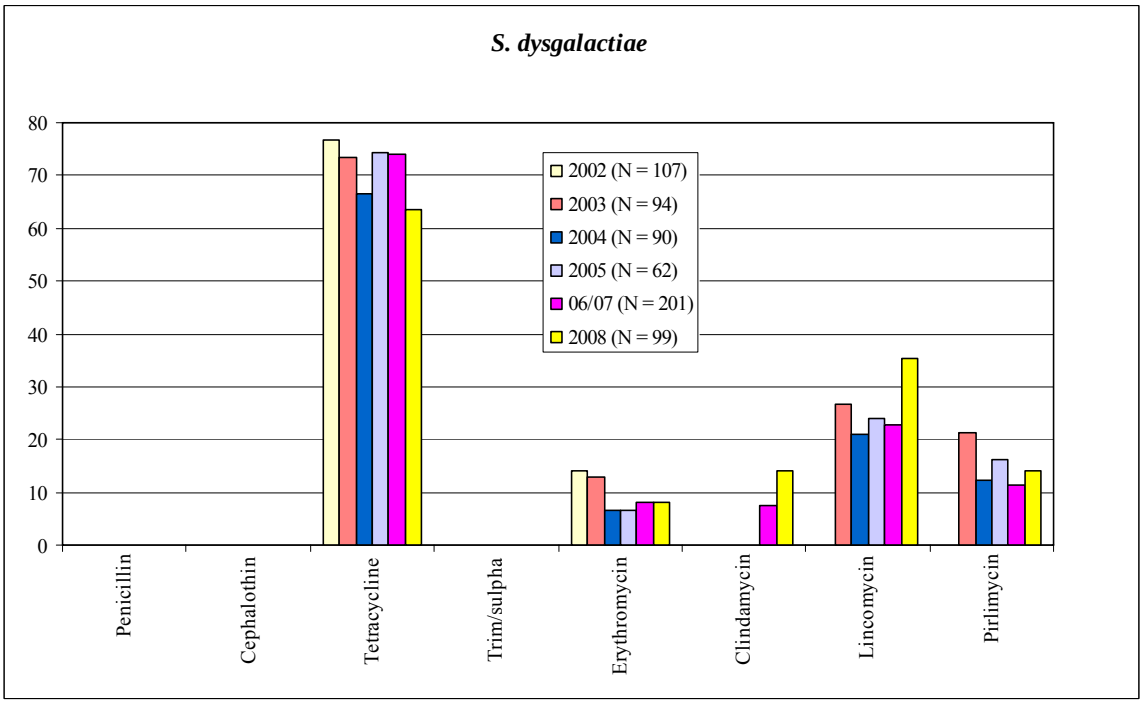
S. dysgalactiae	**MIC % distribution (µg/ml)**															
(99)	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Penicillin	100															0
Cephalothin			1.0	98.0	1.0											0
Erythromycin		7.1	84.8					1.0					7.1			8.1
Clindamycin			53.5	31.3				1.0	7.1				7.1			14.1
Lincomycin				6.1	57.6				1.0	18.2	2.0		15.2			35.4
Pirlimycin			20.2	58.6	4.0	2.0	1.0		6.1	2.0	1.0	1.0	4.0			14.1
Trim/sulpha			39.4	59.6	1.0											0
Tetracycline								6.1	7.1	23.2		1.0	32.3	30.3		63.6

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

In Figure 8.4 the trends in resistance percentages are presented from 2002 to 2008. Although some variation exists, resistance levels seem to be fairly stable. Only in *S. uberis*, resistance against the lincosamides seems to be increasing, with most prominent differences concerning pirlimycin.

Figure 8.4. Trends in resistance percentages for *S. uberis* and *S. dysgalactiae* isolated from milk samples of cattle with intramammary infections in the Netherlands from 2002 - 2008.





III Appendices

Appendix I. Antimicrobial Resistance profiles in animal MRSA in the Netherlands

D.J. Mevius, K.T. Veldman, C.M. Dierikx, H.M. Japing and R. Baaiman

This study was performed in cooperation with the Animal Health Service (GD) in Deventer (P. van der Wolf and A. Rothkamp), the Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM) in Bilthoven (A.W. van de Giessen), the Food and Consumer Product Safety Authority (VWA) in Zutphen (E. de Boer and B. Wit), and the Veterinary Faculty (FD) in Utrecht (H. Graveland, D.J.J. Heederik and J.A. Wagenaar).

After the discovery of the high prevalence of MRSA in pig-, and later also in veal calf farming as well as in persons in close contact with these animals an extensive research program was conducted in the Netherlands to investigate the risk factors for MRSA in Dutch livestock farming. As part of this research program intensive MRSA prevalence studies were performed in different animal species and all isolates were sent to to the Central Veterinary Institute (CVI-Lelystad) for susceptibility testing. The purpose was to determine if next to the *mec*A –gene cluster additional resistance genes had been acquired as a result of different antibiotic use practises in food producing animals.

Methicilline resistance occurs by the presence of the *mec*A gene, which encodes for a variant of the penicilling binding protein (PBP), referred to as PBP2a. PBP2a has reduced affinity for all beta-lactam antibiotics, which prevents beta-lactam antibiotics from disrupting the cell membrane to kill the bacteria. Examples of beta-lactam antibiotics are penicillin, ampicillin, amoxicillin and the combination of amoxicillin and clavulanic acid. Also the so called semisynthetic penicillins like methicilline and oxacillin as well as all cephalosporins belong to this class of antibiotics. This implies that by the presence of the *mec*A gene alone, MRSA has acquired resistance to a large number of important antibiotics. Additionally, MRSA is often resistant against various other antimicrobials like ciprofloxacin, tetracycline, macrolides, gentamicin or clindamycin [1, 2]. Vancomycin resistance in MRSA is rare and so far has not been detected in the Netherlands.

The level of multidrug resistance in MRSA depends on the acquisition of additional resistance determinants and subsequently affects the clinical outcome if MRSA infection is treated with antibiotics.
The prevalence of multidrug resistance is influenced by the use of antibiotics in different settings, for instance in hospitals or in animal husbandry. Antibiotic usage differs between the various food animal production sectors which is reflected in resistance profiles of bacteria from different animal species [3]. Distinctive resistant profiles have already been described in animal related MRSA originating from different animal species [1]. It is important to obtain information on the prevalence and trends in resistance for the purpose of therapeutic advice. The purpose of this study was to identify to determine if next to the *mec*A –gene cluster additional resistance genes had been acquired as a result of different antibiotic use practises in food producing animals.

Materials and methods
In the course of the MRSA study conducted by a consortium of human and veterinary health research institutes and medical centres in the Netherlands, MRSA strains from pigs, calves, food products, other samples from veal calf farms and poultry were sent to the CVI. Isolates were stored at -80°C. Susceptibility testing was performed according to the international reference method ISO 20776-1:2006 for a panel of antimicrobials as shown in Table A1. The MICs were defined as the lowest concentration without visible growth. Strains with MICs higher than the MIC-breakpoints were considered resistant. Percentages of resistance were calculated. Antibiotics in the panel were selected either based on the importance for the treatment of human patients, or on epidemiological grounds to investigate the relation between antibiotic use in animal production and the prevalence of resistance determinants other than the *mec*A gene.
Additionally, a collection of 64 isolates was tested for vancomycine susceptibility using screen plates containing vancomycine in a concentration of 6 mg/L according to CLSI M100 – S20.

Results
Resistance profiles of 1290 MRSA isolates were determined for the antibiotics in the panel. Table A1 shows the MIC distributions for the tested antimicrobials, the breakpoints (cut off values) that were used and the percentage of resistant isolates.
Highest levels of resistance were observed for tetracycline (97%), followed by resistance against erythromycin and clindamycin, the aminoglycosides neomycin and gentamicin, and ciprofloxacin.
Figure A1 shows the resistance according to the sample source. A notable finding is that not all MRSA isolated from poultry and food products are tetracycline resistant. Ciprofloxacin resistance was highest in poultry isolates, while resistance against neomycin and gentamicin is highest in calves. Resistance is low against the combination of

trimethoprim with sulphonamide, a widely used drug in veterinary medicine. Resistance against the antibiotics rifampicine, linezolid and fusidic acid, which are important for the treatment of human patients was either not or only rarely detected.
None of the tested isolates showed resistance against vancomycine.

Table A1. MIC distributions (mg/L) as determined for 1290 animal related MRSA isolates in the Netherlands, concerning 12 antibiotics.

MRSA	MIC (mg/L) distribution (%)												R%
N = 1290	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	
Amikacin			0.2	2.1	26.7	35.7	24.4	9.7	1.2	0.1			1.2
Ciprofloxacin			77.4	6.9	6.9	0.6	1.4	3.7	1.8	1.1	0.2		15.7
Clindamycin		34.2	2.6	0.6		0.1	0.9	1.2	0.6	59.8			62.6
Erythromycin		9.5	27.1	1.2					0.1	62.1			62.2
Fusidic acid		64.4	33.1	1.9	0.1	0.3	0.2	0.1					0.1
Gentamicin			49.6	10.6	2.1	0.9	0.5	1.1	6.7	18.1	10.3		39.8
Linezolid				1.4	45.5	52.9	0.2						0.2
Mupirocin			97.4	1.9	0.7								0
Neomycin			29.5	25.1	11.9	3.5	10.7	9.9	6.4	2.1	0.8		29.9
Rifampin		98.7	0.9	0.2	0.1	0.2							0.2
Trim/sulfa	34.7	5	21.9	27.5	7.8	2.2	0.2	0.1	0.7				3.2
Tetracycline			2	0.7		0.2		0.3	2.2	20.8	73.8		97.1

Discussion
The goal of this study was to determine resistance profiles of MRSA isolated from Dutch food producing animals in order to assess animal or human health risks. For example multidrug resistance or the occurrence of new resistance characteristics are important because of the zoonotic potential of animal related MRSA [2, 4-6].
Most isolates (97%) were **tetracycline** resistant. This was expected as tetracycline resistance is a characteristic for MRSA ST398, based on the presence of a tetM gene [4]. Therefore it was remarkable that also tetracycline susceptible isolates were observed, about 10% of the isolates from poultry and meat products. For the tetracycline susceptible isolates from meat products, it was shown that these belonged to MLST types other than type 398 [7]. No information is available regarding the MLST types of the poultry isolates.

Additionally, high levels of resistance levels were observed for the macrolides as 62,2% of the MRSA isolates had an MIC exceeding 32 mg/l for **erythromycin,** which classified them as resistant. Also high levels of resistance were observed against **clindamycin** (62.6%). Compared to previous findings in pig isolates [8] these data reflect an increase in resistance against lincosamides. This is an important trend which effects the preference of antibiotics for therapeutic treatment in human patients. In hospital settings, clindamycin has been advised as empirical treatment for animal related MRSA infections. This advice was already questioned by Renders et al., which is supported by these data [1].

Ciprofloxacin resistance was commonly found, being highest in MRSA from poultry. This can be explained by the extent of fluoroquinolone use in this animal production sector. Resistance against ciprofloxacin occurs spontaneously owing to point mutations in the chromosome and subsequently, resistant isolates will be selected for when exposed to fluoroquinolones. Antibiotic treatment with fluoroquinolones is quite common in both the veal calf and poultry sector. Moreover, levels of resistance against ciprofloxacin show an increase over the years. In a previous study by the RIVM, all MRSA isolates were still susceptible to this antibiotic [8].

Resistance against the aminoglycosides (**gentamicin** and **neomycin)** showed considerable variation (from 15 to 57%) among the various sample sources. Highest levels of resistance against gentamicin and neomycin were found in veal calves. This has also been described in studies in other countries [1, 4, 8, 9].

Resistance against the trimethoprim/sulphamethoxazole combination was low. This is remarkable, as these antibiotics are widely used for the treatment of food animals. A possible explanation is that clinical resistance requires the presence of two genes (one coding for resistance against trimethoprim together with a gene for resistance against sulphonamides), both of which have rarely been described in *Staphylococcus aureus* [10].

Importantly, animal MRSA isolates showed either no resistance against vancomycine or mupirocin, and sporadic reduced susceptibility to fusidic acid and rifampin, antibiotics that are considered important drugs for the treatment and decontamimation of MRSA in hospital settings. Although some isolates showed MICs just exceeding the cut off value, it is not likely that this reflects the acquisition of specific resistance genes. It is probable that the cut off value is not

entirely adequate to distinguish between the wild type population and strains that have obtained resistance determinants.

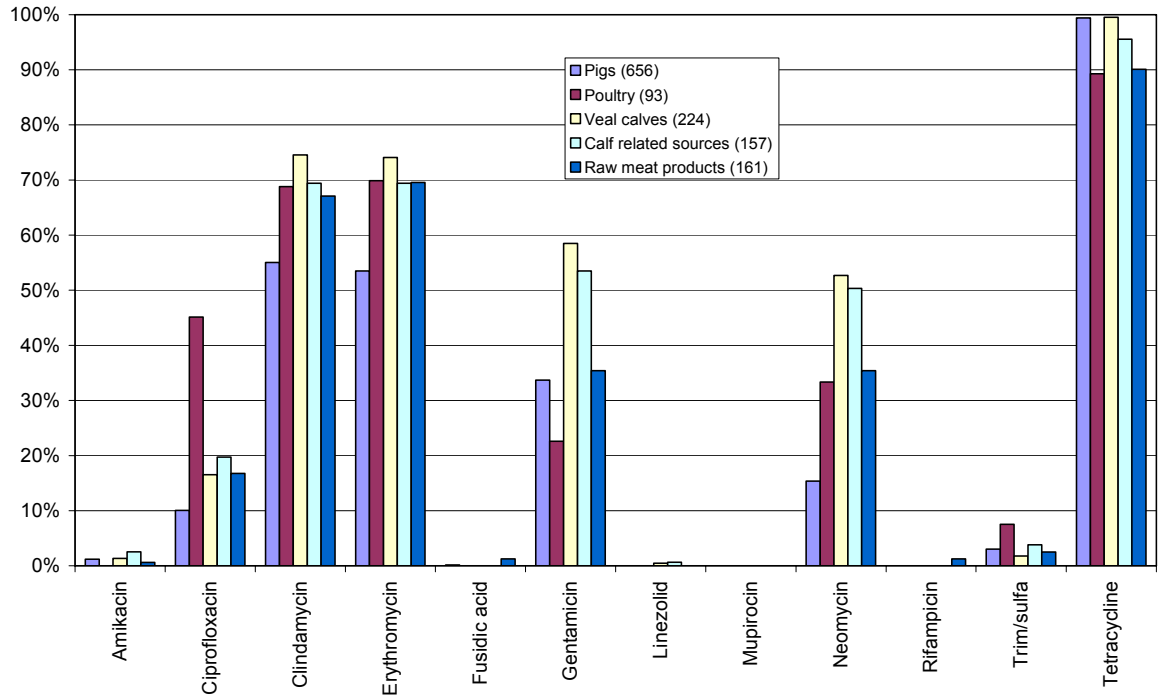
Multidrug resistance was wide spread in animal MRSA. Multiresistance was generally found against beta-lactam antibiotics, macrolides, lincosamides, aminoglycosides (neomycin and gentamicin) and fluoroquinolones.

Recommendations

The data in this study show the importance of surveillance of resistance characteristics of potential zoonotic microorganisms. Based on the widespread prevalence of MRSA in intensive animal husbandry, with its extensive antibiotic use, it is to be expected that the prevalence and extent of antimicrobial resistance will increase in the near future.

Therefore, it is important to continue this surveillance and preferably include other animal species that have been shown to harbour MRSA, like horses, pet animals and dairy cows.

Figure A1. Resistance percentages (%) for animal related MRSA strains isolated from different sample sources in the Netherlands.



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Appendix II. Materials and Methods

Salmonella enterica

A total of 19555 isolates were tested for antimicrobial susceptibility between 1999-2008 (Table A2). Human isolates (N=10630) 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=1459) and cattle, including calves, (N=676) were sent to the RIVM by the Animal Health Service from a diversity of surveillance programs and clinical Salmonella infections. Those from chickens (broilers, including poultry products, N=1452; layers, reproduction animals and eggs, N=864) concerned mainly nonclinical Salmonella infections derived from a diversity of monitoring programs on the farm, slaughterhouses and at retail. The majority of isolates from pigs in 2008 concerned those from the Dutch component of the EU-baseline study. Isolates from a diversity of other sources have been analysed as well (animal fodder and human food products; other animals from animal husbandry and pets, samples from the environment, etc.).

Table A2. Number of Salmonella isolates tested for susceptibility from 1999 – 2008 in the Netherlands.

	Total	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Human	10630	647	349	1055	862	1338	1339	1176	1273	1089	1502
Pig	1459	31	195	114	168	127	119	120	115	135	335
Cattle	676	18	28	56	33	23	106	90	159	87	76
Chicken (misc.)	940	0	10	174	172	160	29	30	116	159	90
Broilers (faeces/meat)	1452	68	110	143	212	206	110	82	164	54	303
Layers/Repro/Eggs	864	93	86	62	56	88	91	232	75	44	37
Other sources	3534	0	9	309	330	446	473	603	535	451	378
Total	19555	857	787	1913	1833	2388	2267	2333	2437	2019	2721

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 A3 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 differ 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 A3. Power analysis to show the sample sizes needed to indicate significant differences in resistance percentages between groups (for example between years or between human and animal sources).

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

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

E. coli and *E. faecium*, *E. faecalis* and *Campylobacter* spp. were isolated from faecal samples taken from healthy animals at slaughter by the Food and Consumer Product Safety Authority as part of the national control programs. Samples were taken at slaughterhouses or at farms. For isolation of the above mentioned organisms one faecal sample was taken for each epidemiological unit (farm, flock or group of animals) aseptically, or the caeca collected (broilers). At the laboratory the samples were directly 1:10 (w/v) diluted in buffered peptone solution with 20% glycerol and stored at –20°C. *E. coli*, *E. faecium*, *E. faecalis* and *Campylobacter* spp. were isolated directly after arrival of the samples at CVI-Lelystad or the Food and Consumer Product Safety Authority in Zutphen. For *E. coli* MacConkey agar and for the enterococci Slanetz and Bartley agar was inoculated with cotton swabs (*E. coli*), or 50 µl of a serial dilution

(enterococci). A colony with typical morphology was subcultured to obtain a pure culture and stored at –80°C in buffered peptone water with 20% glycerol. *E. coli* was identified biochemically. The final identification of the enterococci was done with Polymerase Chain Reaction (PCR) as described by Dutka Malen in 1995[8].
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 program.

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

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

Susceptibility tests
Susceptibility was tested quantitatively with the broth micro dilution test with cation-adjusted Mueller Hinton broth according to ISO standard 20776-1-2006 or CLSI guidelines M31-A3 for Campylobacter spp.. For broth microdilution, microtitre trays were used with dehydrated dilution ranges of custom made panels of antibiotics. Trek Diagnostic Systems, in the UK, manufactured these microtitre trays. ATCC strains *E. coli* 25922 and *E. faecalis* 29212 were used daily to monitor the quality of the results. For quality control of the results of campylobacters, *C. jejuni* ATCC 33560 was used as control strain.
The MICs were defined as the lowest concentration without visible growth. Strains with MICs higher than the epidemiological cut-off values and MIC-breakpoints were considered non-wild type or resistant, respectively. Percentages of resistance were calculated. For *Salmonella*, the indicator organisms *E. coli* and enterococci and *Campylobacter* spp. EUCAST epidemiological cut-off values were used as prescribed by EFSA[9][10] (Table A4). For the animal pathogens clinical breakpoints were used (CLSI M31-A3, M100-S19) as listed in Table A5.

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

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

Table A4. Epidemiological cut-off values (mg/L) used for the classification of *Salmonella*, *E. coli* (indicator organism), *Campylobacter* spp. and enterococci. Isolates with MIC-values higher than those presented in this table are considered resistant.

	Salmonella	*E. coli*	*C. jejuni*	*C. coli*	*E. faecium*	*E. faecalis*
Ampicillin	4	8	8	16	4	4
Cefotaxime	0.5	0.25	-	-	-	-
Ceftazidime	2	0.5	-	-	-	-
Chloramphenicol	16	16	16	16	32	32
Ciprofloxacin	0.06	0.06	1	1	4	4
Clarithromycin	-	-	8	32	-	-
Colistin	8	8	-	-	-	-
Erythromycin	-	-	4	16	4	4
Flavomycin	-	-	-	-	-	16
Florfenicol	16	16	-	-	8	8
Gentamicin	2	2	1	2	32	32
Kanamycin	8	8	-	-	-	-
Linezolid	-	-	-	-	4	4
Nalidixic acid	16	16	16	32	-	-
Neomycin	-	-	1	2	-	-
Quino-dalfopristin	-	-	-	-	1	32
Salinomycin	-	-	-	-	4	4
Streptomycin	32[a]	16	2	4	128	512
Sulphamethoxazole	256[b]	256[b]	256	256	-	-
Tetracycline	8	8	2	2	2	2
Trimethoprim	2	2	-	-	-	-
Tulathromycin	-	-	16	16	-	-
Vancomycin	-	-	-	-	4	4

[a] recommended by EFSA

[b] CLSI breakpoint

Table A5. MIC-breakpoints (mg/L) used for the classification of *E. coli* and coliform bacteria (mastitis), *P. multocida*, *M. haemolytica*, *S. aureus*, coagulase negative staphylococci (CNS) and streptococci. Isolates with MIC-values higher than those presented in this table are considered resistant.

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

Only MecA positive isolates were classified resistant, this equals MIC >4 mg/L