MARAN 2009

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
In 2009

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Colophon

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Usage of antibiotics

The extent to which antibiotics are used for veterinary purposes in food producing animals poses risks to public and animal health. 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 and information about the trend in use per animal species. The results can be used by the Ministry of Economic Affairs, Agriculture and Innovation, that commissioned the study, to measure the effect of policy. Moreover, the usage data can play an important role in explaining trends in resistance that have become apparent. In addition, these data can be used to provide information to the European Commission.

Trends in total sales of antibiotics in the Netherlands
Therapeutic 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 and again slightly decreased in 2009. During this same period, the antimicrobial growth promoters have been banned, first partly from 1999 and as from 2006 entirely. Recent figures indicate that the sales decreased again with 12% in 2010.

Tendencies in exposure to antibiotics in the Netherlands
The figures on exposure to antibiotics in the Netherlands expressed in number of daily dosages per animal per year in the sample surveys reveal the following tendencies for the years 2005-2009, indicating a decrease in antibiotic use in most animal production sectors in 2009:

- sow/piglet farms: annual variation, slight decrease in 2008, increase in 2009;
- fattening pig farms: increased usage from 2005 to 2008, followed by a substantial decrease in 2009;
- broiler farms: increased usage from 2005 to 2008, usage remained stable in 2009;
- veal calf farms: decreased usage from 2007 to 2009;
- dairy farms: increased usage from 2005 to 2008, decrease in 2009.

The usage in fattening pigs in 2008 was statistically significantly higher than the use in 2005. Also the usage in broilers in 2008 was statistically significantly higher than the use in 2005. However, the findings do not permit a distinct conclusion that the use in a particular sector in the Netherlands has increased or decreased, because the observed differences in 2009 were not statistically significant compared to 2008.

Trends in resistance

Salmonella
In 2009, *Salmonella* Enteritidis and *S.* Typhimurium were again the most frequently encountered serovars in human samples, represented by 33.0% and 32.6% of all *Salmonella* sent to RIVM Bilthoven for sero-, and phage typing. *S.* Typhimurium was also frequently isolated in pigs (15.4%) and cattle (17.4%), however was not detected in poultry in 2009. Importantly, the fraction of the multiresistant DT104 has decreased compared to previous years, both in humans and in animals. Instead, *S. enterica subspecies enterica* 1,4,5,12:i:-, as elsewhere in the EU, is strongly emerging, representing 7.0% of all human *Salmonella* isolates, and 11.9% of the porcine isolates. This phage type also shows high levels of resistance, in particular for ampicillin, tetracycline, streptomycin and sulfamethoxazole.
Among animals, *S.* Dublin was again by far the most prevalent serovar in cattle (59.8%) and *S.* Java in poultry (41.5%), especially in broiler chickens. Noteworthy, *S.* Enteritidis was isolated more often in broiler chickens as well as in poultry meat compared to previous years and also *S.* Gallinarum was commonly encountered in poultry, the result of several outbreaks in poultry in the Netherlands in 2009.

Resistance against the for human medicine critically important fluoroquinolones, the first choice antimicrobial for treatment of salmonellosis in humans, was common although differences existed according to serovar or phage type and host species. Overall, 12.3% of human and animal *Salmonella* isolates were reduced susceptible to ciprofloxacin,. Generally, ciprofloxacin resistance occurred more frequently in *S.* Enteritidis than in *S.* Typhimurium, while in *S.* Enteritidis of human origin resistance levels were higher compared to levels in Dutch poultry. The latter finding suggests an additional source of infection for humans (travel, egg imports).
Also resistance against third-generation cephalosporins, critically important antimicrobial for treatment of severe salmonellosis in humans and first choice drug for infected children, was present in *Salmonella* isolates although still at low levels. In 2009, 33 suspected ESBL producing *Salmonella* isolates were found, of which 22 were isolated from animals and meat, 5 from human infections, 1 from spices and 5 were of unspecified origin. Of all ESBL producing isolates, 22 (67%) belonged to *S.* Java, which all originated from poultry.

Campylobacter
Among *C. jejuni* in animal species, highest levels of resistance were detected against tetracycline (45.4%), ampicillin (40.2%) and the quinolones (37.1% and 38.1% for ciprofloxacin and nalidixic acid, respectively). Resistance against tetracyclines varied among different animal species, with very high levels

in veal calves (70% for *C. jejuni* and 96.8% for *C. coli*). Among *C. jejuni* from human origin, 26.5% was resistant against tetracycline. However, 53.2% of the human isolates were resistant against ciprofloxacin, a further increase compared to previous years. In contrast, ciprofloxacin resistance in broilers decreased from 63.3% in 2008 to 42.6% in 2009. Among pigs, the low level of resistance against the quinolones (5.1%) in *C. coli* is consistent with previous years and unlike the high levels of resistance observed in *C. coli* from poultry and cattle (up to 74.2% in veal calves).
It should be noted that mostly disk diffusion is applied at the regional Public Health Laboratories, which may result in discrepancies in resistances levels between human versus animal isolates, as disk diffusion is not standardized and no accepted interpretive criteria are defined.
Resistance against macrolide antibiotics (erythromycin, clarithromycin and tulathromycin) was more commonly observed in *C. coli* (17.7%) than in *C. jejuni* (2.1%). Notable is the high level of resistance in *C. coli* from veal calves, with 25.8% of the isolates resistant against erythromycin, clarithromycin and tulathromycin. Among human isolates, 1.3% was resistant against erythromycin.

Shiga-toxin producing *E. coli* O157 (STEC)
Among human isolates of Shiga-toxin producing *E. coli* O157 resistance levels have been traditionally low. In 2008 an increase was noted, however, levels in 2009 had returned to conventional figures. Highest level of resistance among human isolates was noted for streptomycin and sulfamethoxazole (in 9.5% of the isolates). As expected, resistance in isolates from cattle was more commonly present. Resistance against streptomycin and sulfamethoxazole was seen in 32% and 20% of bovine isolates respectively. Notably resistance was further noted against tetracycline (17.3%). Among Shiga-toxin producing *E. coli* O157 no extended spectrum beta-lactamases (ESBL) suspect phenotypes were present as all human and animal isolates were susceptible to cefotaxime and ceftazidime.

Commensal bacteria *E. coli* and *Enterococcus* species
The level of antimicrobial resistance in commensal bacteria from the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics in animals, especially over time. Resistance levels in commensal bacteria *E. coli* and *Enterococcus* species from pigs, cattle, poultry and their meats are indicative of the occurrence of resistance determinants in the bacterial population in farm animals.

In 2009 in *E coli*, the resistance levels showed a continuous tendency to increase for most antibiotic classes. Notably resistance against beta-lactam antibiotics is disturbing. Resistance against 3rd and 4th generation of cephalosporins, indicative of ESBL producing *E. coli*, was detected in all animal host species, ranging from 1.5% in dairy cattle to 17.9% in broiler chickens.

Overall, enterococci displayed high levels of resistance against tetracycline (92.8% for *E. faecalis*, 71.1% for *E. faecium*) and erythromycin (69.6% for *E. faecalis*, 56.7% for *E. faecium*). Also resistance against streptomycin was common in 2009 (51.0% for *E. faecalis*, 41.9% for *E. faecium*). Remarkable was the increase in resistance against ampicillin among *E. faecium* isolates from pigs, broilers and veal calves.
Vancomycin resistance was observed in low levels in *E. faecium* (1.0%).

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 have decreased. In 2009, there has been a further slight decrease, and recent data indicate a further decrease in 2010. Sample data about the use in specific animal species in the Netherlands indicate a decrease in antibiotic use in three out of five animal production sectors examined in 2009.

Overall, in 2009 the levels of antimicrobial resistance in food-borne pathogens and commensal organisms in the gastro-intestinal tract of food-producing animals are stable or show a tendency to increase. ESBL-suspected *Salmonella* isolates were detected in different sources, but predominantly from broiler chickens. *E. coli* isolates suspected to be ESBL-producing were detected in all food-animal species, but again predominantly in poultry. ESBL-producing *E. coli* was detected in all levels of the poultry production pyramid, indicating vertical transmission.

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 all 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.
- Antimicrobial resistance monitoring remains an essential tool to study the emergence and trends in antimicrobial resistance of animal and, or public health concern.
- The occurrence of *Salmonella* and *E. coli* resistant against third-generation cephalosporins in all food-animals species involved in the monitoring, and the demonstrated genetic relation of these isolates from poultry sources with isolates from infected humans, warrants a continuous monitoring of the genetic characteristics of the organisms in animals and humans.

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 verkoopcijfers van antibiotica voor dieren en inzicht in de trends in gebruik per diersoort. De resultaten kunnen worden gebruikt door het Ministerie van Economische zaken, Landbouw en Innovatie, de opdrachtgever van deze studie, voor het meten van het effect van het beleid. Bovendien kunnen deze gegevens een belangrijke rol spelen in het verklaren van trends in de resistenties in bacteriën uit landbouwhuisdieren. Tevens kunnen de gegevens worden gebruikt voor het informeren van de Europese Commissie.

Trends in de totale verkoop van antibiotica in Nederland
Het therapeutisch 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 en 2009. In dezelfde periode zijn de antimicrobiële groeibevorderaars eerst gedeeltelijk verboden en vanaf 2006 volledig. Uit recente cijfers blijkt dat in 2010 de verkopen verder gedaald zijn, met 12%.

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 2005 tot 2009:

- zeugen/biggen bedrijven: jaarlijkse variatie, afname in 2008, toename in 2009;
- vleesvarkensbedrijven: toename in gebruik van 2005 tot 2008, substantiële afname in 2009;
- vleeskuikenbedrijven: toename in gebruik van 2005 tot 2008, gelijkblijvend gebruik in 2009;
- vleeskalverbedrijven: afname in gebruik van 2007-2009;
- melkveebedrijven: toenemend gebruik van 2005-2008, afname in 2009.

In de meeste sectoren lijkt het gebruik in 2009 te zijn afgenomen. Het gebruik bij vleesvarkens was in 2008 statistisch significant hoger dan het gebruik in 2005. Ook het gebruik bij vleeskuikens was in 2008 statistisch significant hoger dan het gebruik in 2005. Desondanks kan niet met zekerheid worden geconcludeerd dat het gebruik in een bepaalde sector toe- of afgenomen is, omdat de waargenomen verschillen in gebruik tussen 2008 en 2009 niet statistisch significant waren.

Trends in resistentie

Salmonella
In 2009 waren *Salmonella* Enteritidis en *S.* Typhimurium opnieuw de meest voorkomende humane serotypen, vertegenwoordigd door 33,0% en 32,6% van alle *Salmonella* isolaten die in 2009 naar het RIVM in Bilthoven zijn gestuurd voor sero-, en faagtypering. *S.* Typhimurium werd ook regelmatig gevonden bij varkens (15,4%) en rundvee (17,4%), maar werd in 2009 niet bij pluimvee aangetroffen. Opvallend was verder de daling van de fractie van de multiresistente DT104 ten opzichte van voorgaande jaren, zowel bij mens als dier. In plaats daarvan is een opmars te zien van *S.* enterica subspecies enterica 1,4,5,12: i: -, ook in andere Europese landen. Deze monofasische variant betreft bijna altijd *S.* Typhimurium (EFSA expert opinion) en besloeg in 2009 7,0% van alle humane en 11,9% van de varkensisolaten in Nederland. Ook het resistentiepatroon vertoont overeenkomsten, met een hoge mate van resistentie tegen ampicilline, tetracycline, streptomycine en sulfamethoxazole.
Van de isolaten afkomstig van dierlijk monstermateriaal was *S.* Dublin opnieuw veruit de meest voorkomende serovar bij rundvee (59,8%) en *S.* Java bij pluimvee (41,5%), met name vleeskuikens. In vergelijking met voorgaande jaren werd *S.* Enteritidis vaker geïsoleerd, zowel in vleeskuikens als in pluimveevlees. In pluimvee werd verder regelmatig *S.* Gallinarum aangetroffen als gevolg van een aantal uitbraken onder pluimvee in Nederland in 2009.

Regelmatig werd resistentie aangetroffen tegen de antimicrobiële groep van fluoroquinolonen, eerste keuze voor de behandeling van salmonellose bij de mens. Van alle *Salmonella* isolaten vertoonde 12,3% verminderde gevoeligheid voor ciprofloxacin, met grote verschillen tussen serovar of faagtype en diersoort waaruit de *Salmonella* werd geïsoleerd. Over het algemeen kwam ciprofloxacin resistentie vaker voor in *S.* Enteritidis dan in *S.* Typhimurium, terwijl in *S.* Enteritidis van humane oorsprong hogere resistentieniveaus werden gevonden in vergelijking met die uit Nederlands pluimvee. Deze laatste bevinding duidt op een alternatieve bron van infectie voor de mens (reizen, ei-importen).
Hoewel nog steeds op een laag niveau werd ook in 2009 resistentie aangetroffen tegen de derde en vierde generatie cefalosporinen, indicatief voor ESBL producerende salmonella's. In totaal werden 33 cefotaxime resistente stammen gevonden , waarvan er 22 afkomstig waren uit dieren of vlees, vijf uit humaan monstermateriaal, één uit een kruidenmonster, terwijl van vijf de oorsprong niet was vermeld. Van

alle ESBL verdachte isolaten behoorden 22 (67%) tot S. Java. Voor zover de herkomst van deze stammen bekend was waren ze alle afkomstig uit pluimvee.

Campylobacter
Hoogste resistentiepercentages in *Campylobacter* uit dierlijk monstermateriaal werd gevonden tegen tetracycline (45,4%), ampicilline (40,2%) en de quinolonen (37,1% voor ciprofloxacin en 38,1% voor nalidixinezuur). Resistentie tegen tetracycline verschilde per diersoort, met hoogste resistentie in vleeskalveren (70% voor *C. jejuni* en 96,8% voor *C. coli*). Onder *C. jejuni* uit humaan monstermateriaal was 26,5% tetracycline resistent. Van de humane isolaten vertoonde 53,2% resistentie tegen ciprofloxacin, een verdere stijging ten opzichte van voorgaande jaren. Daarentegen daalde de ciprofloxacin resistentie bij vleeskuikens van 63,3% in 2008 naar 42,6% in 2009.
Opnieuw werd in *C. coli* een opvallend laag resistentiepercentage gezien tegen de quinolonen bij varkens (5,1%) in vergelijking met de percentages in *C. coli* uit pluimvee en rundvee (tot 74,2% in vleeskalveren).
Resistentie tegen macrolide antibiotica werd vaker waargenomen in *C. coli* (17,7%) dan in *C. jejuni* (2,1%). Opvallend is het hoge resistentiepercentage in *C. coli* uit vleeskalveren, waarbij 25,8% van de isolaten resistentie vertoonden tegen erythromycine, claritromycine en tulathromycine. Daarentegen was 1,3% van de humane isolaten resistent tegen erythromycine.
Overigens dient vermeld te worden dat de humane streeklaboratoria voornamelijk gebruik maken van de disk diffusie methode. Aangezien de disk diffusie niet is gestandaardiseerd en goed gedefinieerde interpretatieve criteria ontbreken, kan dit resulteren in verschillen in resistentiepercentages tussen humane en dierlijke isolaten,.

Shiga-toxine producerende *E. coli* O157 (STEC)
Resistentiepercentages van Shiga toxine producerende *E. coli* O157 uit humaan materiaal zijn traditioneel laag. Na een onverklaarbare stijging in 2008, waren de resistentiepercentages in 2009 weer als vanouds. Hoogste resistentiepercentages werden gezien voor streptomycine en sulfamethoxazole (beide in 9,5% van de humane isolaten). Zoals verwacht werd resistentie vaker gezien in isolaten van runderen, met name tegen streptomycine (32%), sulfamethoxazole (20%) en tetracycline (17,3%). Onder Shiga-toxine producerende *E. coli* O157 werden geen ESBL verdachte isolaten gevonden.

Commensale bacteriën *E. coli* en *Enterococcus* species
Het voorkomen van antimicrobiële resistentie bij commensale bacteriën uit het darmkanaal weerspiegelt de selectiedruk als gevolg van het gebruik van antibiotica in dieren, met name in de tijd. Resistentiespercentages in *E. coli* en *Enterococcus* species van varkens, rundvee, pluimvee en hun vlees worden gebruikt als indicatie van het vóórkomen van resistentie determinanten in de bacteriële populatie in landbouwhuisdieren.

In *E. coli* is sinds een aantal jaren sprake van een toename in resistentie voor de meeste antibiotica klassen. Deze trend zet zich voort in 2009. Met name de toename in resistentie tegen beta-lactam antibiotica is verontrustend. Cefotaxime resistentie, indicatief voor ESBL producerende *E. coli* werd aangetroffen bij alle voedselproducerende diersoorten, variërend van 1,5% in melkvee tot 17,9% bij vleeskuikens.
De enterokokken, afkomstig uit voedselproducerende dieren en vlees, lieten een hoge mate van resistenties zien tegen tetracycline (92,8% in *E. faecalis*, 71,1% in *E. faecium*) en erythromycine (69,6% voor *E. faecalis*, 56,7% voor *E. faecium*). Ook resistentie tegen streptomycine werd vaak gezien (51,0% voor *E. faecalis*, 41,9% voor *E. faecium*). Opvallend was verder de toename van de resistentie tegen ampicilline bij *E. faecium*-isolaten van varkens, vleeskuikens en vleeskalveren.
Vancomycine resistentie werd incidenteel gevonden in *E. faecium* (1,0%).

Conclusies en aanbevelingen

Na een periode van voortdurende toename van het therapeutisch antibioticagebruik bij landbouwhuisdieren in Nederland, is de totale hoeveelheid verkochte antibiotica, uitgedrukt per kg levend gewicht, in 2008 en 2009 gedaald. Ook de steekproefgegevens over het antibioticagebruik in specifieke diersoorten laten in drie van de vijf onderzochte dierlijke productiesectoren in 2009 een daling van het gebruik zien.

Over het algemeen was in 2009 het niveau van antimicrobiële resistentie bij ziektekiemen in voedsel en commensale organismen in het maag-darmkanaal van voedselproducerende dieren stabiel of vertoonde een stijgende tendens. ESBL-verdachte *Salmonella*-isolaten waren afkomstig uit verschillende bronnen, maar voornamelijk uit vleeskuikens. Ook *E. coli* isolaten waarvan vermoed wordt dat ze in staat zijn tot ESBL-productie werden gezien in alle voedselproducerende diersoorten, maar met name bij pluimvee. ESBL-producerende *E. coli* zijn aangetroffen in alle niveaus van de pluimveeproductie piramide, duidend op verticale transmissie.

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 alle 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.

- In het kader van de volksgezondheid blijft monitoring van antimicrobiële resistentie een essentieel instrument om inzicht te krijgen in het ontstaan en de ontwikkeling van resistentie bij dieren.
- Het voorkomen van *Salmonella* en *E. coli* met resistentie tegen derde-generatie cefalosporinen in alle voedselproducerende diersoorten en de bewezen genetische verwantschap van pluimvee isolaten met isolaten uit humane infecties, rechtvaardigt een continue monitoring van de genetische kenmerken van de organismen in zowel dier als mens.

I Usage of antibiotics in animal husbandry in the Netherlands

1. Introduction

Aim

The objective of this study is to obtain detailed insight into the exposure of farm animals to antibiotics, by monitoring both sales data at the national level and more specifically data per animal species. The results from the study can be used by the Ministry of Economic Affairs, Agriculture and Innovation to measure the effect of policy. In addition, the usage data can play an important role in explaining trends in resistance that have become apparent. Moreover, these data can be used to inform the European Commission.

Monitoring in the Netherlands

Since 1998 FIDIN, the federation of the Dutch veterinary pharmaceutical industry, annually reports antibiotic sales figures in the Netherlands (FIDIN, 2010). The sales figures stated in the reports give an impression of the total amount of active substances used in the Netherlands at the level of pharmacotherapeutic groups. Moreover, the monitoring of antibiotic use on a stratified sample of Dutch farms provides information about the true exposure of farm animals to antibiotics, and into the underlying factors that could explain changes in antibiotic use. The use in veal calves has been monitored in an additional sample, in cooperation with the veal calf sector.

Monitoring in Europe

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. Therefore the European Medicines Agency (EMA) is carrying out the ESVAC project to establish national systems for the collection of data on sales of veterinary antimicrobial agents in Europe, in a standardized way (EMA, 2010). ESVAC stands for European Surveillance of Veterinary Antimicrobial Consumption. The project was set up in April 2010 to collect information on how antimicrobial medicines are used in animals across the European Union. The EMA started this project following a request from the European Commission for the Agency to develop a harmonised approach for the collection and reporting of data on the use of antimicrobial agents in animals from the EU Member States. EC Directive 2001/82/EC and Regulation 726/2004 provide a legal basis for national authorities to request the pharmaceutical industry to provide data on sales of antimicrobial agents. However, member states are not yet obliged to provide data about the use of veterinary antibiotics to the EC.

Table 2.1. Trends in livestock in the Netherlands in numbers (thousands)[1]

Number of animals * 1.000	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Piglets (less than 20 kg)	4,791	4,935	4,422	4,225	3,896	4,300	4,170	4,470	4,680	4,555	4,809
Sows	1,320	1,272	1,161	1,140	1,052	1,125	1,100	1,050	1,060	1,025	1,100
Fattening pigs (of at least 50 kg)	4,668	4,560	5,911	5,772	5,807	5,705	5,715	5,880	5,960	6,135	6,192
Other pigs	2,360	2,055	20	17	11	10	15	-180	10	20	7
Turkeys	1,544	1,544	1,523	1,451	1,112	1,238	1,250	1,200	1,250	1,250	1,356
Other poultry	54,506	54,506	48,642	48,714	43,168	44,427	45,800	45,475	45,050	46,958	48,865
Veal calves	800	756	676	692	748	775	813	824	860	913	886
Cattle (veal calves excluded)	3,297	3,134	3,166	3,088	2,986	2,984	2,933	2,849	2,960	3,083	3,112
Sheep	1,152	1,250	1,250	1,300	1,476	1,700	1,725	1,755	1,715	1,545	1,099

Source: Eurostat 2010

[1] Other pigs is a calculated number (statistical adjustment).

Table 2.2. Trends in livestock in the Netherlands in live weight (tonnes)

kg live weight *1.000	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Pigs	831,204	793,152	715,992	699,434	678,786	691,691	685,943	675,876	699,092	703,127	725,258
Turkeys	9,264	9,264	9,138	8,706	6,672	7,428	7,500	7,200	7,500	7,500	8,135
Other poultry	54,506	54,506	48,642	48,714	43,168	44,427	45,800	45,475	45,050	46,958	48,865
Veal calves	141,040	133,283	119,179	122,000	131,943	136,633	143,332	145,271	151,618	160,962	156,202
Cattle (veal calves excluded)	1,648,500	1,567,000	1,583,000	1,544,000	1,493,150	1,492,000	1,466,500	1,424,500	1,480,000	1,541,500	1,556,000
Sheep	69,120	75,000	75,000	78,000	88,560	102,000	103,500	105,300	102,900	92,700	65,940
Total	**2,753,634**	**2,632,205**	**2,550,951**	**2,500,854**	**2,442,279**	**2,474,179**	**2,452,575**	**2,403,622**	**2,486,160**	**2,552,747**	**2,560,401**

2. Materials and methods

2.1 Method used for analysis of sales data

The FIDIN report presents 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 information about 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, poultry, veal calves, other cattle, sheep). For this analysis the following average weights have been used: veal calves (weighted average of white and rosé) 176 kg, other cattle 500 kg, turkeys 6 kg, other poultry 1 kg, fattening pig 70 kg, sow 220 kg, other pigs 70 kg, piglets (< 20 kg) 10 kg, sheep 60 kg. 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 yearly average numbers of animals present in the Netherlands (Table 2.1) are converted into live weight (Table 2.2). To obtain insight into the trends in antibiotic usage based on the national sales data, the total antibiotic sales are divided by the total live weight of the average present livestock (see Figure 3.1).

2.2 Method used for analysis of usage data on farms in the Farm Accountancy Data Network

This report reviews the antibiotic usage in 2009, and is based on a total of 231 pig, broiler and dairy cattle farms in the Farm Accountancy Data Network. The results about veal calves are based on 193 farms in a large additional sample. See Table 2.3 for details.

The Data Network contains a representative sample of around 1,500 agricultural and horticultural farms in the Netherlands (Vrolijk et al, 2009). 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. On these farms all animal-medicine data and veterinary services are recorded. The data for the veal calves originate from an additional aselect sample of all veal calf farms. On these farms detailed data were collected on number of animals present and the amount of antibiotics used.

These data are available on the websites of LEI (www.lei.wur.nl and www.maran.wur.nl) and CVI (www.cvi.wur.nl). They provide further insight into the use of antibiotics expressed as the number of daily dosages per animal year and about the use in grams of active ingredients per animal year. Note that the data only apply to the farms in the sample(s). The aggregated usage data with 95% confidence intervals are considered to be representative for the total exposure of Dutch food-producing animals to antibiotics. The 95% confidence intervals indicate 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 given.

Table 2.3. Number of sample farms taking part each year and the associated number of animals

Type of holding		2005	2006	2007	2008	2009
Number of animals	Sows/piglets	16790	12155	17949	18767	20806
	Fattening pigs	54788	55970	119922	156098	159104
	Broilers	2367623	2315882	2197716	2508103	2530313
	Veal calves	n.a.	n.a.	124096	134437	134446
	Dairy cows	2875	2818	2850	7274	7382
Number of farms	Sows/piglets	46	32	39	45	48
	Fattening pigs	40	31	49	77	72
	Broilers	29	28	29	29	28
	Veal calves	n.a.	n.a.	185	199	193
	Dairy cows	35	34	34	82	83
Total		150	125	336	432	424

n.a. = no data available

2.3 Unit of measurement: number of daily doses per animal year

To provide insight into the true exposure of animals to antibiotics the use is expressed in the number of daily doses per animal year: ADD (Jensen et al, 2004). Antibiotics vary in their potency and pharmacokinetic properties[2], and this results in different dosages per kilogram body weight between and within antibiotic classes. Because of these differences the unit "grams per kg live weight", as calculated from total sales figures, is a less accurate indicator for the use of antibiotics. The unit "daily dosage" is more suitable for calculating the exposure of animals to antibiotics. Adopting this approach offers an opportunity to obtain more insight into the relationship with the occurrence and trends in 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 to compare 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 average present livestock on the farm[3]. 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 enables 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. The use of antibiotics in spray containers is not included.

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 at the time of 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 true exposure, especially for piglets, fattening pigs and veal calves.

[2]Differences in dosage are determined by differences in potency, differences in bioavailability and distribution throughout the body.

[3]This is the average weight of the animals (in kg per animal) multiplied by the average number of animals present on the farm per year.Note that on dairy farms only the weight of the dairy cows has been taken into account.

In the sample survey the following average weights have been used: dairy cows 600 kg, veal calves 176 kg (i.e. the weighted average of white veal calf 164 kg and rosé veal calf 192 kg), broilers 1 kg, fattening pigs 70.2 kg, sows 220 kg, maiden gilts 107.5 kg, piglets (< 25 kg) 12.5 kg, breeding boars 350 kg (ASG, 2010). On dairy farms the number of daily dosages is based on the weight of the dairy cows only, because this category of the animals gets almost all of the antibiotics. However, on sow farms the size of the 'population at risk' is based on the weight of all present animals (including piglets, gilts, breeding boars).For an illustrative calculation of the number of daily dosages for young calves on dairy farms (from birth to weaning at 56 days of age) the average weight of 56.5 kg has been used.

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[4]. 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 (X contains 40% = 400 mg/ml active ingredient a) and 20 kg of antibiotic preparation Y (Y contains 25% = 250 mg/g active ingredient b). Antibiotic preparation X: the defined daily dosage of active ingredient a is 10 mg per kg animal weight per day. Antibiotic preparation Y:the defined daily dosage of active ingredient b is 50 mg per kg animal weight per day.

Antibiotic X can be used to treat (2,000 * 400)/10 = 80,000 kg animal weight. Antibiotic 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[5] on the farm in that year was administered a prescribed dosage of antibiotics on 17.1 days. In this example the farm uses 17.1 daily dosages per animal year of antibiotic preparation X plus Y.

[4] For veal calves the calculated daily dosages are based on the highest allowed dosage instead 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.

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

2.4 Statistical analysis

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

This limited number of farms used may affect the validity of the conclusions drawn for the whole population. However, the selection of farms in the sample should be representative for the different animal production sectors. In this way a sample can provide adequate information (Vrolijk et al, 2009). 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.

To ensure that the farms that are included in the sample are representative of the whole population and to make the sampling as efficient as possible a disproportional stratified random sampling strategy is used. A stratified sample implies that the population is divided into a number of homogeneous groups. Subsequently farms are selected from each of the groups. For strata with larger variation in the use of antibiotics, relatively more sample farms are selected. In the FADN sample the strata are based on both farm size and animal category. The additional sample of veal calf farms is additionally stratified for 'large integration' versus 'small integration or non-contracted farms'.
Since the stratification is disproportional, the results have to be weighted to be representative. For each stratum the average daily dosages per animal year is determined. Then the weighted average for an animal category is calculated, based on the number of farms in the population in each stratum.

In this report the usage data of all 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[6] 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.

[6] See Appendix 3 in Vrolijk et al. (2008) for more detail about the statistics.

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, 2010). Table 3.1 shows the use specified per group of antibiotics, including the use of antimicrobial growth promoters (AGP).

Figure 3.1. Veterinary therapeutic antibiotic sales from 1999-2009 (FIDIN, 2010; vertical bars). The line presents the trends in grams of active ingredients used per kg live weight.

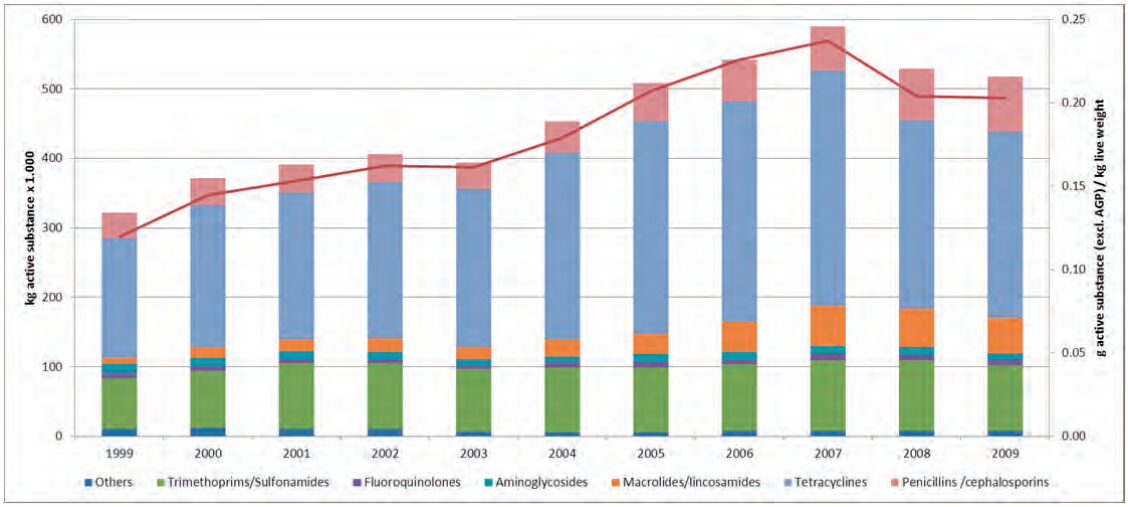


Figure 3.1 reveals that the total amount of antibiotics sold by the pharmaceutical industry in the Netherlands for therapeutic veterinary use has increased in the period 1998-2007. Compared to 2007 in 2008 the amount of antibiotics sold has decreased by 10% and in 2009 by a further decrease of 2%. Recently FIDIN reported a further reduction of 12% of veterinary antibiotic sales in 2010, to 455 tonnes. We consider this reduction to be the result of the efforts of the animal production sectors and the authorities that take part in the Task Force Antimicrobial Resistance in Animals.

The use of antimicrobial growth promoters was prohibited at the beginning of 2006. Apart of the increase of therapeutic antibiotic use in the years 1998-2006 may be accounted for by a substitution of growth promoters. See Table 3.1 for the quantitative figures about the use of AGP.

Over the years, the number of the livestock has also changed. The trends in therapeutic antibiotic use in relation to the number and weight of animals in the Netherlands are presented in Figure 3.1.

Table 3.1. Antibiotic sales from 1999-2009 in tonnes (FIDIN, 2010)

	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Penicillins /cephalosporins	37	38	40	40	38	45	54	60	64	74	79
Tetracyclines	171	204	211	225	227	269	307	317	338	271	268
Macrolides	10	16	18	20	18	24	29	44	58	55	51
Aminoglycosides	13	12	11	10	9	9	11	11	12	11	10
Fluoroquinolones	7	7	6	6	5	7	8	7	9	8	8
Trimethoprim/sulfonamides	73	82	94	94	90	93	93	95	101	102	95
Other	11	12	11	11	7	6	6	8	8	7	8
Total therapeutic use	322	371	391	406	394	453	508	542	590	529	518
Antimicrobial growth promoters (AGP)	250	205	180	140	120	80	40	0	0	0	0
Antibiotic use including AGP	572	576	571	546	514	533	548	542	590	529	518

3.2 Trends in exposure to antibiotics in the Netherlands

Figure 3.2 shows the tendencies in exposure to antibiotics in defined daily dosages per average animal present per year (ADD) in the five sectors examined in this study, based on the farms in the samples. The outcome of the calculations is indexed, using 2009 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 3.2.

Figure 3.2 Tendencies in relative antibiotic usage from 2005-2008 in percentages daily dosages per animal year at the sample farms in 2005-2009 (daily dosages per animal year in 2009 = 100%). For veal calves data are available as from 2007.

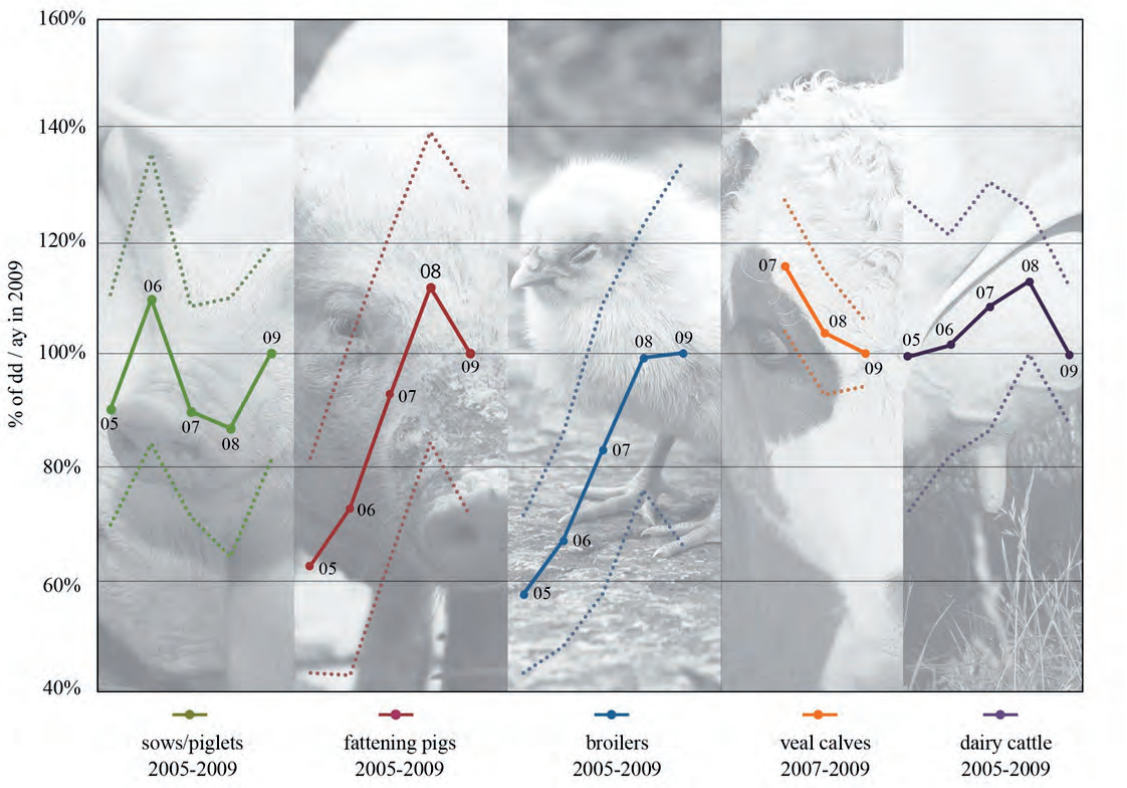


Figure 3.2 shows different tendencies in exposure to antibiotics in the different animal species, indicating a decrease in antibiotic use in most animal production sectors in 2009:

- sow/piglet farms: annual variation, slight decrease in 2008, increase in 2009;
- fattening pig farms: increased usage from 2005 to 2008, followed by a substantial decrease in 2009;
- broiler farms: increased usage from 2005 to 2008, usage remained stable in 2009;
- veal calf farms: decreased usage from 2007 to 2009;
- dairy farms: increased usage from 2005 to 2008, decrease in 2009.

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

It is important to note that in spite of the variation presented, the data in figure 3.2, representing the use of antibiotics at the farms in the sample, do not permit a distinct 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 less variation, strengthened by a relatively large sample size. The monitoring in the veal calf sector started in 2007.

4. Antibiotic usage per animal species in 2009

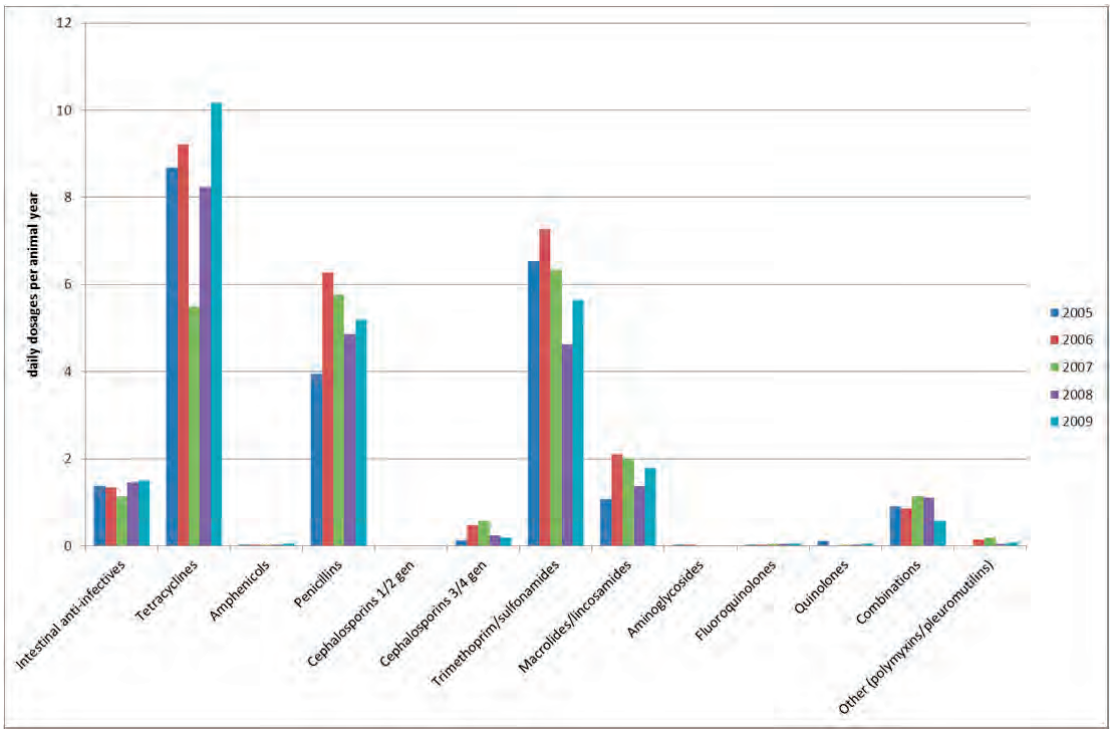
4.1 Pigs

Sows and piglets

In 2009, the average animal on sow/piglet production farms in this survey received approximately 25 daily dosages per year. More than 80% was orally administered, probably predominantly in piglets. The average use in the sow/piglet sector in the Netherlands will be at most 19% higher or lower than the average determined at the sample farms (95% Confidence Interval: 21-30 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 into the trends in the relative use of the various groups of antibiotics on the sample farms

Figure 4.1. Antibiotic use in sows/piglets in daily dosages per sow per year in 2005-2009



In 2009 40% of the total antibiotic use in sows/piglets consisted of tetracyclines, 22% of trimethoprim/sulfonamides and another 21% of penicillins. 0.7% of the total usage consisted of 3rd and 4th generation cephalosporins and 0.2% consisted of fluoroquinolones, that are likely to have been administered to young piglets before weaning.

Discussion

We observe a tendency to reintroduce conservative therapy with traditional antibiotic groups (tetracyclines, trimethoprim/sulfonamides) instead of introducing newer antibiotics like cephalosporins, fluoroquinolones and macrolides.

For sows/piglets in the sample the average use in 2009 was 25 daily dosages per year. However, in practice almost all of the antibiotics are likely used for the treatment of the piglets, and only incidentally for the sows. If it is assumed that 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 treated with antibiotics during 25 days in the period from birth to the age of 74 days (at delivering to the fattening farm, at 25 kg).

The findings reveal a positive correlation between the size of sow farms and the antibiotic use per animal, as shown in table 4.1 below. Note that the figures in the table are mean values. On a quarter of the farms with more than 250 sows the usage of antibiotics was under 20 daily dosages per animal year.

Table 4.1 Number of daily dosages per animal year for different farm sizes

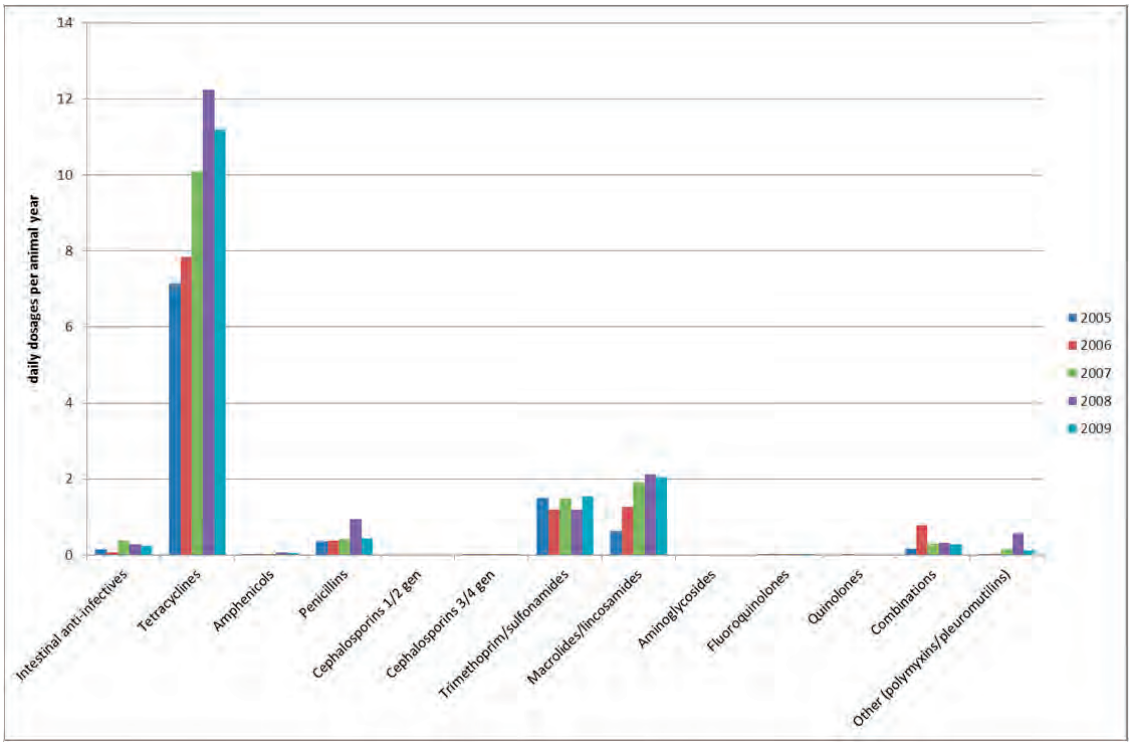
	< 250 sows	250-600sows	> 600 sows
Daily dosages per animal year	15	30	50

Fattening pigs

The average fattening pig in the sample received 16 daily dosages per year in 2009, of which nearly 97% orally administered. The average use on pig fattening farms in the Netherlands will be at most 28% higher or lower than the average determined at the sample farms (95% Confidence Interval: 11-20 dd/ay).

Figure 4.2 provides insight into the trends in the relative use of the various groups of antibiotics on the sample farms

Figure 4.2. Antibiotic use in fattening pigs in daily dosages per animal year in 2005-2009



In 2009 70% of the total antibiotic use in fattening pigs originated from the administration of tetracyclines and 13% from macrolides/lincosamides. Cephalosporins and (fluoro)quinolones were not applied.

Discussion

The average fattening pig in the sample was treated with antibiotics 16 days per year. Assuming a production period of 117 days, 5 daily dosages (= 16 x (117/365) 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 (during 25 days), which brings the total exposure to antibiotics per average fattening pig to approximately 30 days during its whole life from birth to slaughter at the average age of 191 days.

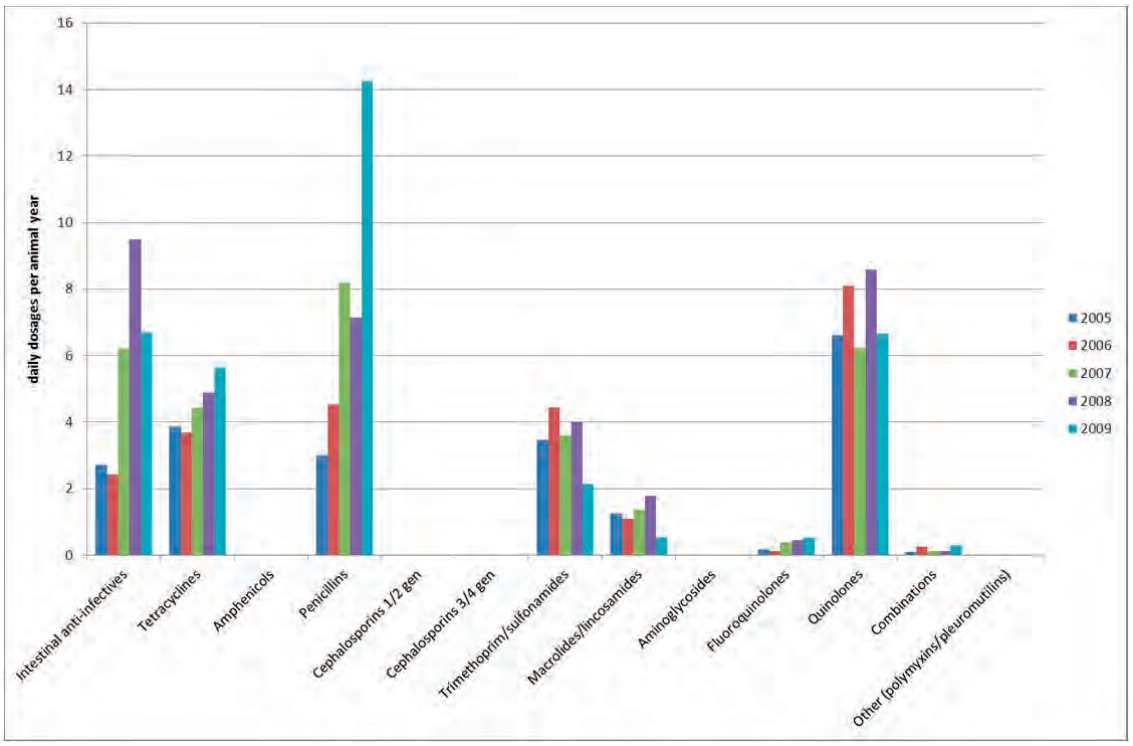
If it is assumed that the average treatment weight of fattening pigs will be thirty percent lower than their average live weight, since younger animals are more likely to receive antibiotics than older animals, the estimation of the total life time true exposure increases from 30 days to a total of 32 days.

4.2 Broilers

The average boiler chicken in the sample received 37 daily dosages per year in 2009, administered orally, mainly through the drinking water. Based on the 95% Confidence Interval of 24-49 dd/ay the average use on broiler farms in the Netherlands will be at most 34% higher or lower than the average determined at the sample farms.

Figure 4.3 provides insight into the trends in the relative use of the various groups of antibiotics on the sample farms.

Figure 4.3 Antibiotic use in broilers in daily dosages per animal year in 2005-2009



In 2009 administration of penicillines accounted for 39% of the total antibiotic use on broiler farms, intestinal anti-infectives for 18%, quinolones for 18% and tetracyclines for 15%. Fluoroquinolones use was 1.4% of the total use. The use of intestinal anti-infectives (e.g. neomycin, colistin) decreased from 9.5 dd/ay in 2008 to 6.7 dd/ay in 2009. In the same period the use of penicillins almost doubled from 7.2 in 2008 to 14.3 in 2009.

Discussion
On the average broiler farm in the sample 37 daily dosages of antibiotics are administered per year. This means that an individual broiler is treated with antibiotics during 4 days (= 37 x 42/365) in the 42 days from day one to slaughter.

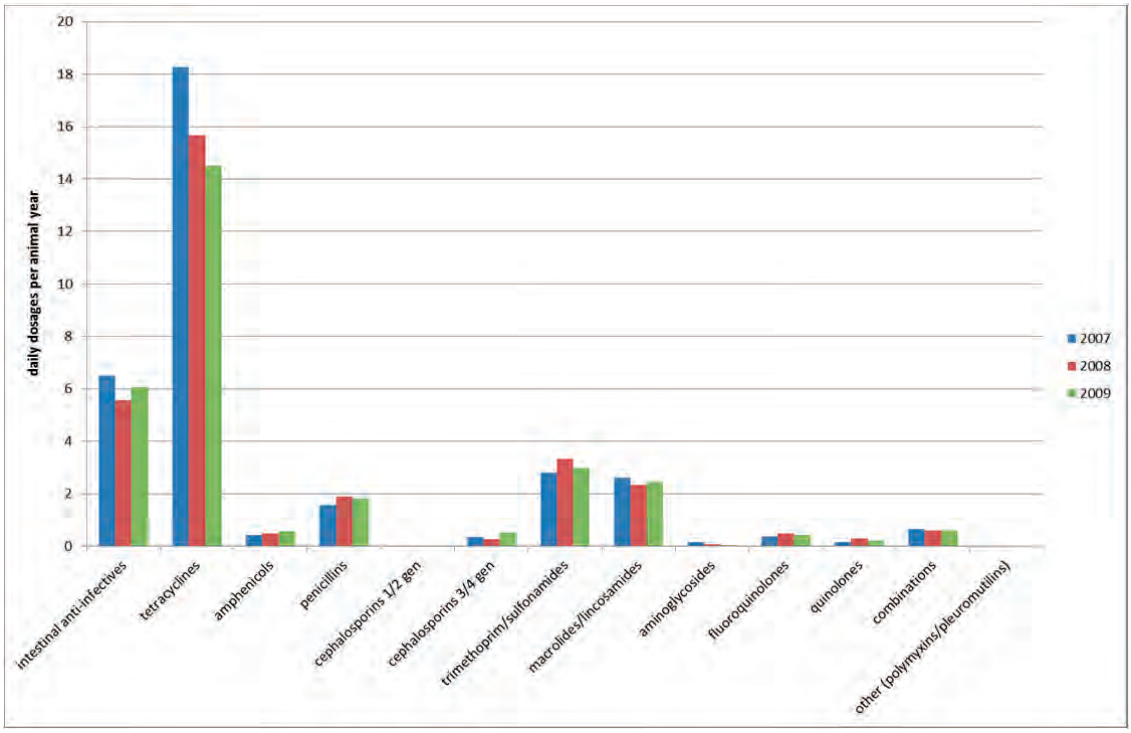
Data on the time of prescription reveal that the average weight on which broilers receive treatment equals the average live weight of 1.0 kg. Therefore the calculated exposure of approximately 4 days per broiler can be considered as an adequate estimation of the true exposure (i.e. 3 to 6 treatment days per broiler, considering the 95% confidence interval).

4.3 Veal calves

The average veal calf in the sample received 30 daily dosages per animal year in 2009, of which more than 90% was orally administered. The average use on veal calf farms in the Netherlands will be at most 6% higher or lower than the average determined at the sample farms (95% Confidence Interval: 28-31dd/ay).

Figure 4.4 provides insight into the trends in the relative use of the various groups of antibiotics on the sample farms.

Figure 4.4. Antibiotic use in veal calves in daily dosages per animal year in 2007-2009



In 2009 49% of the total antibiotic use on veal calf farms originated from the administration of tetracyclines, 20% from intestinal anti-infectives (e.g. neomycin, colistin) and 10% from trimethoprim/sulfonamides. Fluoroquinolones and 3rd and 4th generation cephalosporin use were both 1.3% of the total use.

Discussion

The overall use decreased, mainly as a result of less traditional antibiotic therapy with tetracyclines. The application of the newer antibiotics like cephalosporins, fluoroquinolones and macrolides seems to be unchanged, whereas a decrease was expected.

On the average veal calf farm in the sample 30 daily dosages of antibiotics are administered per year. This means that the individual average veal calf is treated with antibiotics during 18 days (= 30 x 222/365) in the period from birth to the average slaughter age of 222 days (white and rosé).

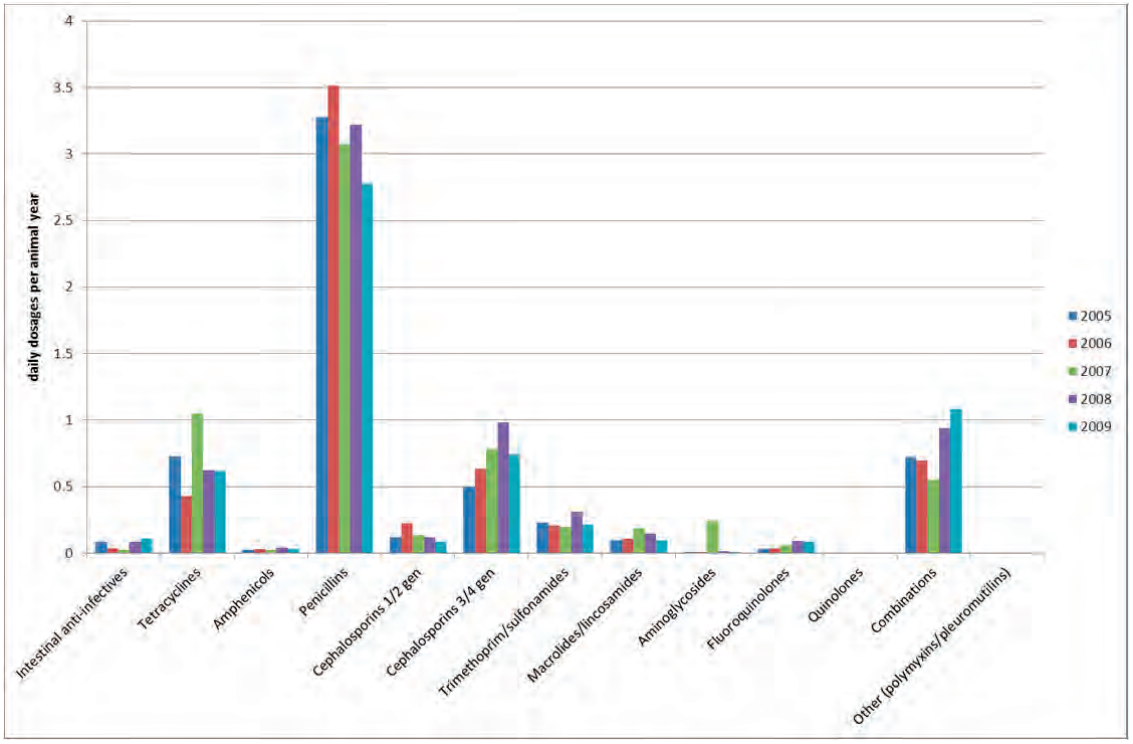
If it is assumed that the average treatment weight of veal calves is about fifty percent lower than the average live weight, since younger animals are more likely to receive antibiotics than older animals, the estimation of the total life time true exposure increases from 18 days to a total of 36 days.

4.4 Dairy cows

The average dairy cow in the sample received 5.8 daily dosages per year in 2009, including the use in young stock. The average use on dairy farms in the Netherlands will be at most 12% higher or lower than the average determined at the sample farms (95% Confidence Interval: 5.1-6.5 dd/ay).

Figure 4.5 provides insight into the trends in the relative use of the various groups of antibiotics on the sample farms.

Figure 4.5. Antibiotic use in dairy cattle in daily dosages per animal year in 2005-2009



In 2009 48% of the total antibiotic use on dairy farms originated from the administration of penicillins and 19% from combinations, which were mainly applications for intramammary treatment. Also 13% third/fourth generation cephalosporins were used. Use of fluoroquinolones is limited in dairy cattle.

Discussion

After a couple of years of steadily increasing application of the newer antibiotics like 3rd and 4th generation cephalosporins, fluoroquinolones and macrolides, 2009 may represent a turning point.

On the average dairy farm in the sample in 2009 5.8 daily dosages of antibiotics were administered per year, of which 3.4 for intramammary use and 0.3 for oral use. If it is assumed that the oral use is only applied in young calves, an average calf is exposed to antibiotics during 9 days of the 56 day weaning period. Note that part of the antibiotics that are registered for oral treatment may have been used off-label in footbaths for disinfection of cattle feet instead of treating sick calves. 68% of the intramammary use is used for drying off, which means that on average 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. On the basis of monitoring at a sample of farms there is also more detailed information available 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 and 2009. Recent sales figures indicate a further decrease of 12% in 2010. 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
Figure 3.2 shows different tendencies in exposure to antibiotics in the different animal species for the years 2005-2009, indicating a decrease in antibiotic use in most animal production sectors in 2009:

- sow/piglet farms: annual variation, slight decrease in 2008, increase in 2009;
- fattening pig farms: increased usage from 2005 to 2008, followed by a substantial decrease in 2009;
- broiler farms: increased usage from 2005 to 2008, usage remained stable in 2009;
- veal calf farms: decreased usage from 2007 to 2009;
- dairy farms: increased usage from 2005 to 2008, decrease in 2009.

The usage in fattening pigs in 2008 was statistically significantly higher than the use in 2005. Also the usage in broilers in 2008 was statistically significantly higher than the use in 2005. However, the findings do not permit a distinct conclusion that the use in a particular sector in the Netherlands has increased or decreased, because the observed differences in 2009 were not statistically significant compared to 2008.

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Annexes

Annexes to Part I Usage of antibiotics in animal husbandry in the Netherlands are available on the MARAN website at www.maran.wur.nl.

II Resistance data

In this chapter susceptibility test results are presented as determined in 2009 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.*

6. Food-borne pathogens

Salmonella

Resistance percentages are presented on *Salmonella* 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 2009, *Salmonella* Enteritidis and *S.* Typhimurium were again the most frequently encountered serovars in human samples, represented by 33.0% and 32.6% of all *Salmonella* sent to RIVM Bilthoven for sero-, and phagetyping, followed by *S. enterica subspecies enterica* 1,4,5,12:i:- (7.0%). This serovar, as elsewhere in the EU, is strongly emerging, while the relative portion of DT104 has decreased compared to previous years.
As in previous years, *S.* Dublin was by far the most prevalent serovar in cattle (59.8%), followed by *S.* Typhimurium (17.4%). In pigs, *S.* Typhimurium was still the most common serovar in 2009 (15.4%), although its prevalence seems to be decreasing over the years. In 2009, *S. enterica subspecies enterica* 1,4,5,12:i:- was commonly isolated, represented by 11.9% of the porcine isolates.
S. Java is still the most important *Salmonella* serovar in poultry, especially in broiler chickens. In 2009, 41.5% of all *Salmonella* isolates from poultry were identified as such. Noteworthy, *S.* Enteritidis was isolated more often in broiler chickens as well as in poultry meat compared to previous years. Also *S.* Gallinarum was commonly encountered in 2009, the result of several outbreaks that have occurred in poultry in the Netherlands.
The resistance levels in Salmonella isolates against the clinically important fluoroquinolones, the first choice antimicrobial for treatment of salmonellosis in humans, differed among serovars and phage types. Generally, ciprofloxacin resistance was higher in *S.* Enteritidis than in *S.* Typhimurium. As in previous years, in *S.* Enteritidis of human origin resistance levels were higher compared to levels in Dutch poultry, suggesting an additional source of infection (travel, egg-imports).
Also resistance against third-generation cephalosporins, the second choice antimicrobials for treatment of severe *Salmonella* infections in humans, was present in Salmonella isolates, although still at low levels. In 2009, 33 suspected ESBL producing *Salmonella* isolates were found, of which 22 were isolated from animals and meat, 5 from human infections, 1 from spices and 5 were of unspecified origin. Of all ESBL producing isolates, 22 (67%) belonged to *S.* Java either derived from poultry or from an unspecified source.

Salmonella prevalence

In 2009 a total of 2124 *Salmonella* isolates were sent to RIVM Bilthoven for sero-, and phagetyping, of which 1901 were tested for antimicrobial susceptibility (Table 6.1 and 6.2). Human isolates (N=1112) 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=227) and cattle (N=92), including calves 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=188; layers, reproduction animals and eggs, N=50) concerned mainly nonclinical *Salmonella* infections derived from a diversity of monitoring programs on farms, slaughterhouses and at retail. A large proportion of isolates from pigs in 2009 concerned those from the Dutch component of the EU-baseline study in breeding pigs. Isolates from a diversity of other sources have been analyzed as well (animal feed and human food products; other animals from animal husbandry and pets, samples from the environment, etc.).

Table 6.1. Most prevalent *Salmonella* sero-, and phage types isolated in 2007/2008 and 2009 from humans, pigs, poultry, broilers and layers and the % travel related human infections from 2007 - 2009.

	Travel	Humans		Pigs		Cattle		Poultry		Broilers		Layers	
	>2007	07/08	2009	07/08	2009	07/08	2009	07/08	2009	07/08	2009	07/08	2009
N total	7%	2910	1220	1540	227	198	92	1139	347	809	188	136	50
N tested		2653	1112	480	53	170	73	721	221	514	106	89	39
Typhimurium	3%	31,3	32,6	22,0	15,4	23,2	17,4	4,0		3,3		7,4	
ft507	1%	9,7	8,2	4,2	5,3	6,6	4,3	1,1		1,1			
ft506 (DT104)	2%	8,3	5,1	4,0	3,1	5,6	3,3	1,1		0,9		2,9	
ft651	2%	0,7		0,2		0,5	1,1	0,3				1,5	
ft353	1%		2,0		0,4		1,1						
ft561 (DT7)	0%	0,7	0,7	0,3	0,4	0,5							
ft90	0%		1,7					0,1					
Enteritidis	15%	35,4	33,0	2,7	3,5	2,0	1,1	8,3	14,4	4,8	12,2	28,7	28,0
Pt4	8%	8,8	7,4	0,4	0,4			2,5	2,9	1,1	3,2	11,0	2,0
Pt21	18%	6,1	4,1	0,1		0,5		0,9	4,6	0,6	4,3	2,2	12,0
Pt8	12%	6,3	5,7	0,6	1,3	1,0	1,1	1,7	1,2	1,2	1,1	2,2	4,0
Pt1	21%	3,2	3,3	0,3				0,4	0,6	0,1	1,1	1,5	
Pt6	21%	2,9	4,1	0,1				0,2	0,3	0,2			
Pt14b	19%	1,5	2,0	0,1				0,2		0,1		0,7	
Agona	29%	0,4	0,3	1,6	1,3			1,1	0,3	1,1		0,7	
Anatum	23%	0,4		2,2	1,3	0,5		0,5	0,9	0,5	1,6		
Bovismorbificans	5%	0,4	0,2	2,1	5,7	1,5		0,3		0,1		1,5	
Brandenburg	2%	0,5	0,3	5,5	3,5	0,5		0,1	0,6				2,0
Corvallis	29%	0,8	0,8					0,1	0,3	0,1			
Derby	7%	0,4	0,7	15,3	7,9	0,5		0,2	0,6	0,1	0,5	0,7	2,0
Dublin	3%	0,4	0,2	0,3	0,4	62,6	59,8						
Gallinarum	--							2,1	4,6			16,2	20,0
Hadar	25%	0,4	0,3	0,5				1,8	1,4	2,3	2,1		
Heidelberg	9%	0,5	0,8	0,6				1,8	1,4	1,4	1,1	2,2	4,0
Infantis	14%	0,9	2,8	4,2	6,2			9,5	5,8	9,6	4,8	3,7	8,0
Paratyphi B var Java	7%	0,7	0,7	0,2		1,0		46,9	41,5	56,6	55,9	1,5	
Kentucky	33%	1,3	1,1	0,1				0,3		0,2			
Livingstone	6%	0,2	0,2	4,4	4,8			1,0	1,7	0,6	1,1	2,9	2,0
London	4%	0,3	0,4	8,6	4,8	0,5	1,1						
Mbandaka	17%	0,4	0,2	0,5	0,9		1,1	3,2	3,5	4,2	5,9	1,5	
Montevideo	24%	0,4	0,3	0,6	0,4	4,0	2,2		0,6		1,1		
Newport	23%	1,3	2,0	0,3	0,4			1,1		0,6			
Oranienburg	19%	0,8	0,2	0,1				0,1	0,6			0,7	4,0
Rissen	28%	0,0	0,2	0,8	2,2			0,3		0,1		0,7	
Saintpaul	28%	0,7	0,4	0,1				0,4		0,6			
Senftenberg	26%	0,4	0,2	0,5	1,3		1,1	1,8	1,7	1,5	1,6	3,7	2,0
Tennessee	8%	0,1	0,4	0,1	0,4			0,6		0,4		2,9	
Thompson	9%	0,3	0,2	0,3				1,3	1,2	0,6	0,5	5,9	
Virchow	34%	1,5	1,2	0,3				2,3	0,3	2,1		5,1	
Weltevreden	25%	0,5	0,3										
Goldcoast	2%	0,3	0,2	4,6	2,2								
Fluntern	--							0,4				3,7	
Panama	6%	1,4		0,9	0,9			0,1		0,1			
SI 1,4,5,12:i:-	2%	5,6	7,0	6,4	11,9	0,5	7,6	1,8	0,9	1,5	0,5		
(Para)Typhi (A B C)	31%	1,5	2,0										
Other	--	10,4	10,3	14,3	24,2	3,0	8,7	8,9	17,9	7,3	11,2	10,3	28,0

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

As in previous years, *S.* Enteritidis and *S.* Typhimurium were the most prevalent serovars isolated from humans in the Netherlands (Table 6.1), represented by 33.0% and 32.6% of all isolates respectively. Third was again the antigenic monophasic variant of *S.* Typhimurium: *S. enterica subspecies enterica* 1,4,5,12:i:- (7.0%), followed by *S.* Infantis. This *Salmonella* serovar showed a noteworthy increase from 0.9% in 2007/2008 to 2.8% in 2009. Both serovars are also recovered from animal sources, although their relative prevalence varies considerably over the years.

Traditionally, *S.* Typhimurium has been the most detected serovar in pigs. The relative presence seems to be decreasing over the years, in 2009 15.4% of all *Salmonella* isolates from pigs consisted of *S.* Typhimurium serovars. Other commonly found serovars in 2009 were *S. enterica subspecies enterica* 1,4,5,12:i:- (11.9%), *S.* Derby (7.9%), *S.* Infantis (6.2%), *S.* Bovismorbificans (5.7%), *S.* Livingstone and *S.* London (both 4.8%).

As in previous years, *S.* Dublin was by far the most prevalent serovar in cattle (59.8%), followed by *S.* Typhimurium (17.4%).

In poultry, *S.* Paratyphi B var. Java (*S.* Java) was again the most predominant serovar overall. In 2009, *S.* Java was only recovered from samples from broiler chickens represented by 55.9% of all isolates. In layers, as before, the leading serovar in 2009 was *S.* Enteritidis (28% of all *Salmonella* isolates). The second most prevalent serotype among layers in 2009 was *S.* Gallinarum (20.0%), which has been implicated in several outbreaks in the Netherlands since the fall of 2009.

In 2008 we reported a striking decrease in *S.* Senftenberg prevalence among layers compared to previous years (from 24.9% to 1.7%). In 2009, its contribution was similar to 2008 (2.0%).

From 2007 onwards travel contributed up to 34% of the cases of human salmonellosis depending on the sero/phagetype. A substantial contribution was noted for the serovars Virchow (34%), Kentucky (33%), and typhoidal *Salmonella* cases (31%). However, several other serovars regularly are related to travel abroad. It should be noted that the contribution of travel as depicted in Table 6.1 is only indicative of the true contribution, because travel is estimated to be underreported by about a factor two.

Table 6.2. MIC distribution (in %) and resistance percentages (R%) for all *Salmonella*'s (N = 1901) tested for antibiotic susceptibility in 2009

Salmonella N = 1901	MIC (%) distribution mg/L																	R%	95% CI
	0.015	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Ampicillin						2.1	46.0	27.2	2.9	0.2	0.1	0.1	21.5						**21.8**	19.9 - 23.7
Cefotaxime			47.3	43.7	6.3	0.9	0.1			1.6									**1.7**	1.2 - 2.4
Ceftazidime					68.3	27.9	2.1	0.1	0.5	0.1	0.2	0.8							**1.6**	1.1 - 2.3
Gentamicin					14.5	63.0	19.1	1.8	0.2	0.2	0.3	0.6	0.4						**1.6**	1.1 - 2.3
Kanamycin									91.8	5.2	0.6	0.1	0.1		2.2				**2.9**	2.2 - 3.8
Streptomycin								2.4	20.1	20.6	28.0	9.4	3.7	4.7	11.0				**19.5**	17.7 - 21.3
Tetracycline							3.0	65.0	9.6	0.5	0.2	1.9	3.3	16.4					**21.8**	20.0 - 23.8
Sulfamethoxazole										51.2	22.3	1.9	0.1	0.1		0.1	0.3	24.0	**24.4**	22.5 - 26.4
Trimethoprim						86.1	1.3	0.2		0.1			12.5						**12.5**	11.1 - 14.1
Ciprofloxacin	24.8	59.8	3.2	0.9	6.4	2.4	1.3	0.2	0.1	0.5	0.5								**12.3**	10.9 - 13.9
Nalidixic acid									82.4	4.7	1.7	0.2	0.3	10.8					**11.3**	9.9 - 12.8
Chloramphenicol									13.9	73.2	7.0	0.4	0.2	5.3					**5.9**	4.9 - 7.1
Florfenicol								0.4	41.5	49.5	3.6	0.8	2.3	1.8					**5.0**	4.1 - 6.1
Colistin										100									**0**	0 - 0.2

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

Resistance levels

Table 6.2 presents MIC-distributions and resistance percentages of all *Salmonella*'s tested for susceptibility in 2009 (n = 1901). Highest levels of resistance were observed for sulfamethoxazole, ampicillin, tetracycline, streptomycin and to a lesser extend trimethoprim, ciprofloxacin, and nalidixic acid.
Resistance profiles vary considerably among serovars as shown in Table 6.3. This table presents resistance percentages for the fourteen most prevalent serovars isolated in The Netherlands in 2009. Highest resistance levels are observed in *S.* Typhimurium, *S.* Java, the monophasic *S. enterica subspecies enterica* 1,4,[5],12:i:-, *S.* Kentucky and *S.* Virchow.
In *S.* Java characteristic findings are high level resistance against trimethoprim which is characteristic of the clone, in combination with acquired resistance against the quinolones and third generation cephalosporins cefotaxime and ceftazidime.

ESBL's
In 2009, 33 cefotaxime reduced susceptible (MIC > 0.5 mg/L) ESBL suspected isolates were found. The isolates belonged predominantly to the serovar *S.* Java (67%, N = 22). Of 17 of these *S.* Java isolates the origin was reported, all of which were recovered from poultry. Other ESBL-suspected serovars were *S.* Heidelberg and *S.* Cerro recovered from poultry products; *S.* Enteritidis (Pt 13a), *S.* Haifa, *S.* Infantis, *S. enterica subspecies enterica* 1,4,5,12:i:-, and *S.* Typhimurium (ft506) from humans, *S.* Krefeld from spices and *S.* Virchow from minced meat. Eleven of these isolates (33%) were resistant against nalidixic acid and also showed reduced susceptibility to ciprofloxacin (MIC 0.25 – 2 mg/L). 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 (see appendix 1).

Quinolone resistance
Using the epidemiological cut off value of 0.06 mg/L, 234 *Salmonella* isolates (12%) were detected that demonstrated a non-wild type phenotype for ciprofloxacin. Of these, 24 (1.3%) showed MICs larger that the clinical breakpoint (1 mg/L). The serovars of these ciprofloxacin resistant isolates were predominantly travel related *S.* Kentucky (67%). Other serovars were *S.* Java from poultry (8.3%), *S.* Cholerasuis (8.3%), *S.* Virchow (4.1%), *S.* Krefeld (4.1%), *S.* Agona (4.1%) and *S.* Blockley (4.1%). Since 2002 annually high-level ciprofloxacin resistant *S.* Kentucky's were isolated from human patients as noted in other EU countries as well. 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 2009.

	Enteritidis (413)	Typhimurium (378)	subsp.enterica 1,4,[5],12:i:- (104)	Paratyphi B var Java (96)	Infantis (73)	Dublin (62)	Senftenberg (41)	Livingstone (35)	Anatum (33)	Newport (29)	Mbandaka (28)	Kentucky (25)	Virchow (21)	Derby (20)
Ampicillin	7.5	49.2	77.9	61.5	2.7	1.6	2.4	11.4	3.0	0	0	28.0	19.0	20.0
Cefotaxime	0.2	0.3	1.0	22.9	1.4	0	0	0	0	0	0	0	4.8	0
Ceftazidime	0.2	0	1.0	22.9	0	0	0	0	0	0	0	0	4.8	0
Gentamicin	0.2	0	2.9	6.3	1.4	0	0	2.9	0	0	0	40.0	4.8	0
Kanamycin	0.2	0.8	3.8	10.4	5.5	0	0	11.4	0	0	0	4.0	0	5.0
Streptomycin	2.2	38.4	82.7	43.8	11.0	1.6	0	11.4	3.0	3.4	3.6	48.0	19.0	25.0
Tetracycline	4.4	46.3	86.5	21.9	12.3	3.2	2.4	11.4	0	6.9	0	48.0	33.3	25.0
Sulfamethoxazole	1.7	55.3	85.6	62.5	15.1	0	2.4	17.1	0	3.4	7.1	44.0	28.6	35.0
Trimethoprim	0.5	22.5	7.7	93.8	9.6	0	2.4	11.4	3.0	0	3.6	0	28.6	35.0
Ciprofloxacin	13.3	5.6	1.9	51.0	15.1	4.8	4.9	2.9	0	0	0	64.0	66.7	0
Nalidixic acid	12.8	4.8	1.0	46.9	15.1	4.8	4.9	2.9	0	0	0	64.0	61.9	0
Chloramphenicol	0.2	22.2	4.8	6.3	4.1	0	0	2.9	0	0	0	0	0	5.0
Florfenicol	0	21.4	1.0	2.1	2.7	0	2.4	0	0	0	0	0	0	0
Colistin	0	0	0	0	0	0	0	0	0	0	0	0	0	0

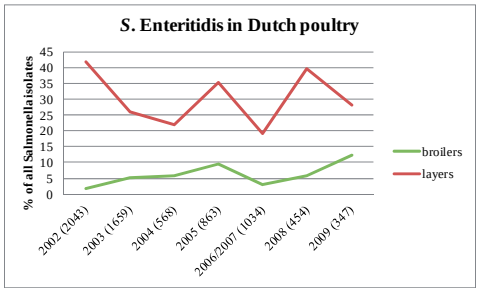
S. Enteritidis

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 (12.2% and 17.1% respectively) is substantially lower than *S.* Java (55.9% and 62.2% respectively) as shown in Tables 6.1 and 6.7. Although *S.* Enteritidis prevalence varies over the years, it is traditionally higher in layers than in broiler chickens (Figure 6.1)

The difference in phagetypes isolated from Dutch broilers and humans and the moderate resistance of strains from human infections compared to the lack of resistance in Dutch layers indicates that other sources of infection exist amongst them travel (Table 6.1) and imported eggs.

In Table 6.4 and Figure 6.2, resistance percentages and multidrug resistance for *S.* Enteritidis are specified according to host and most prevalent phage types. In 2009, no resistance was observed in *S.* Enteritidis isolated from layers, while resistance levels in *S.* Enteritidis recovered from human samples and broilers were low.

Figure 6.1. Salmonella Enteritidis prevalence among broilers en layers in the Netherlands. Between brackets are the total number of poultry isolates sent to the RIVM per year.



The most commonly recovered phage type in 2009 was still Pt4 (24.7%), followed by Pt8 (18.2%) and Pt21 (15.3%). Resistance profiles showed some variation among the different phage types, most notably with respect to the quinolone antibiotics nalidixic acid and ciprofloxacin. Highest resistance levels were observed in Pt1 and Pt14b that are often travel related in humans.

Table 6.4. Resistance (%) of *S.* Enteritidis isolated from different sources and phage types 4, 8, 21, 6, 1, and 14b in 2009.

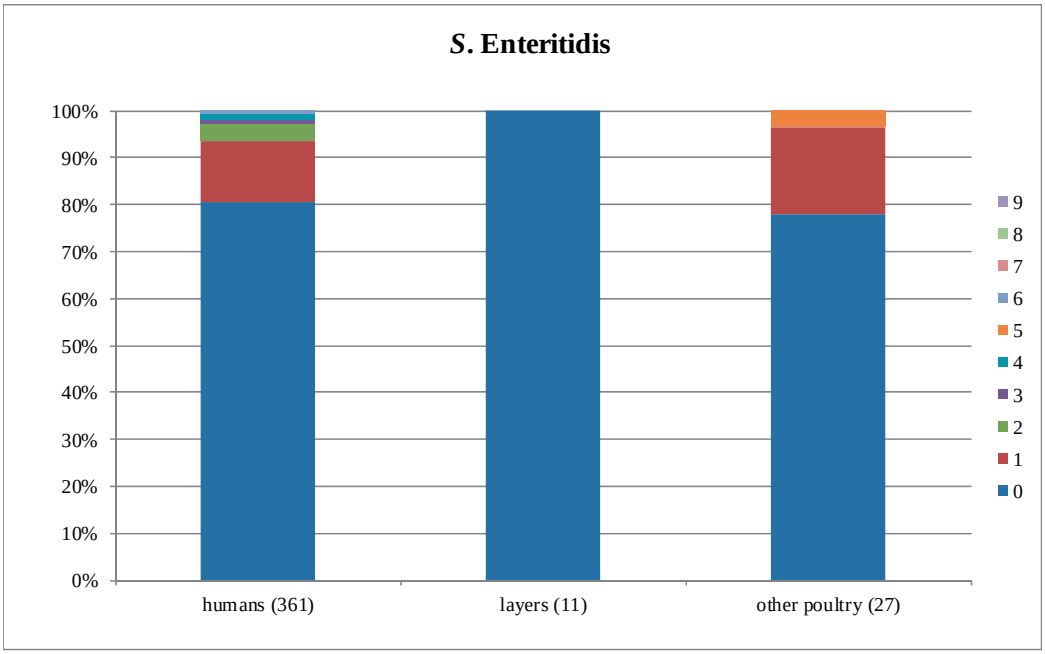
	S. Enteritidis			Most prevalent phage types					
	Humans (361)	Layers (11)	Other poultry (27)	Pt4 (102)	Pt8 (75)	Pt21 (63)	Pt6 (44)	Pt1 (40)	Pt14b (25)
Ampicillin	7.5	0	14.8	2.0	0.0	6.3	9.1	5.0	12.0
Cefotaxime	0.3	0	0	0	0	0	0	0	0
Ceftazidime	0.3	0	0	0	0	0	0	0	0
Gentamicin	0.3	0	0	0	0	0	0	2.5	0
Tetracycline	4.7	0	3.7	1.0	0	6.3	6.8	5.0	8.0
Sulfamethoxazole	1.7	0	3.7	1.0	0	4.8	0	5.0	0
Trimethoprim	0.6	0	0	0	0	1.6	0	0	0
Ciprofloxacin	14.4	0	11.1	4.9	1.3	6.3	9.1	55.0	24.0
Nalidixic acid	13.9	0	11.1	4.9	1.3	6.3	9.1	55.0	20.0
Chloramphenicol	0.3	0	0	0	0	1.6	0	0	0
Florfenicol	0	0	0	0	0	0	0	0	0
Streptomycin	2.2	0	3.7	1.0	0	3.2	0	5.0	8.0
Kanamycin	0	0	0	0	0	0	0	2.5	0
Colistin	0	0	0	0	0	0	0	0	0

Quinolone resistance

In 2009, 51 ciprofloxacin non wild type susceptible strains were isolated from human infections, predominantly Pt1 (35%), to a lesser extend Pt14b (12%), Pt6a (12%), Pt4 (10%), Pt21 (8%) and Pt6 (8%). Although Dutch poultry may have contributed to the ciprofloxacin non-wild type/nalidixic acid resistant isolates found in humans, the vast majority of the human infections 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.
In isolates from human infections the resistance levels remained stable, while in isolates from poultry annual variation is much more apparent (Fig. 6.3). After a remarkable peak in resistance against the quinolones in 2005 and 2006/2007, related to the relative proportion of Pt4 and Pt1, levels have decreased to low levels again. It should be noted however, because of the small numbers of isolates each year, that any conclusions on trends must be drawn with great care.

Figure 6.2. Percentages of *S.* Enteritidis strains fully susceptible, resistant to one to nine different antibiotic classes in human and animal sources in the Netherlands in 2009, presented by source (top figure) or phage type (lower figure).



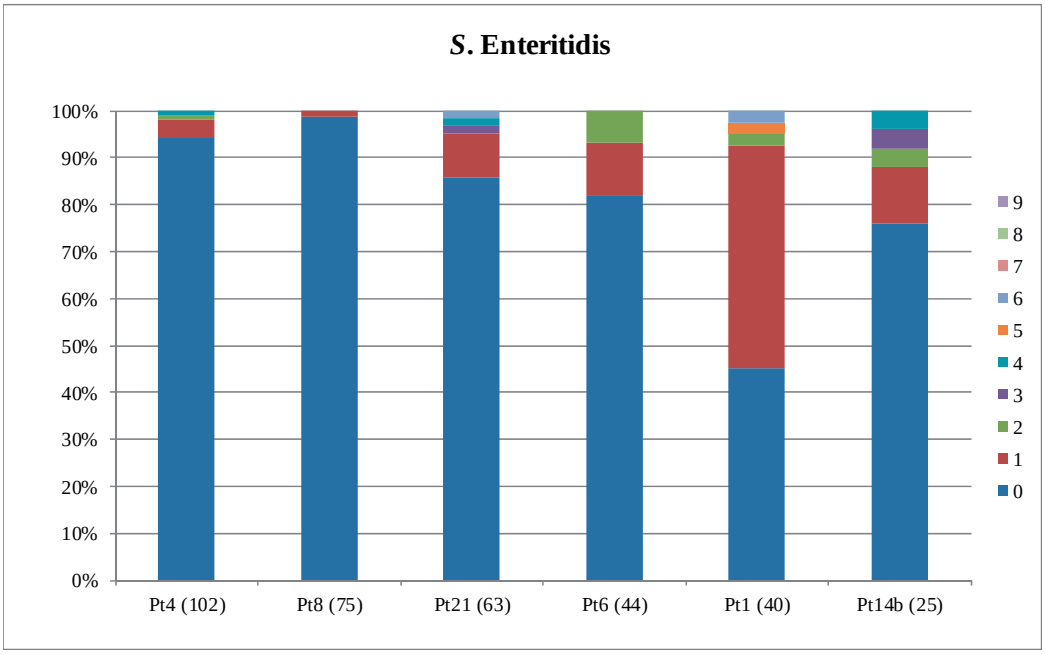
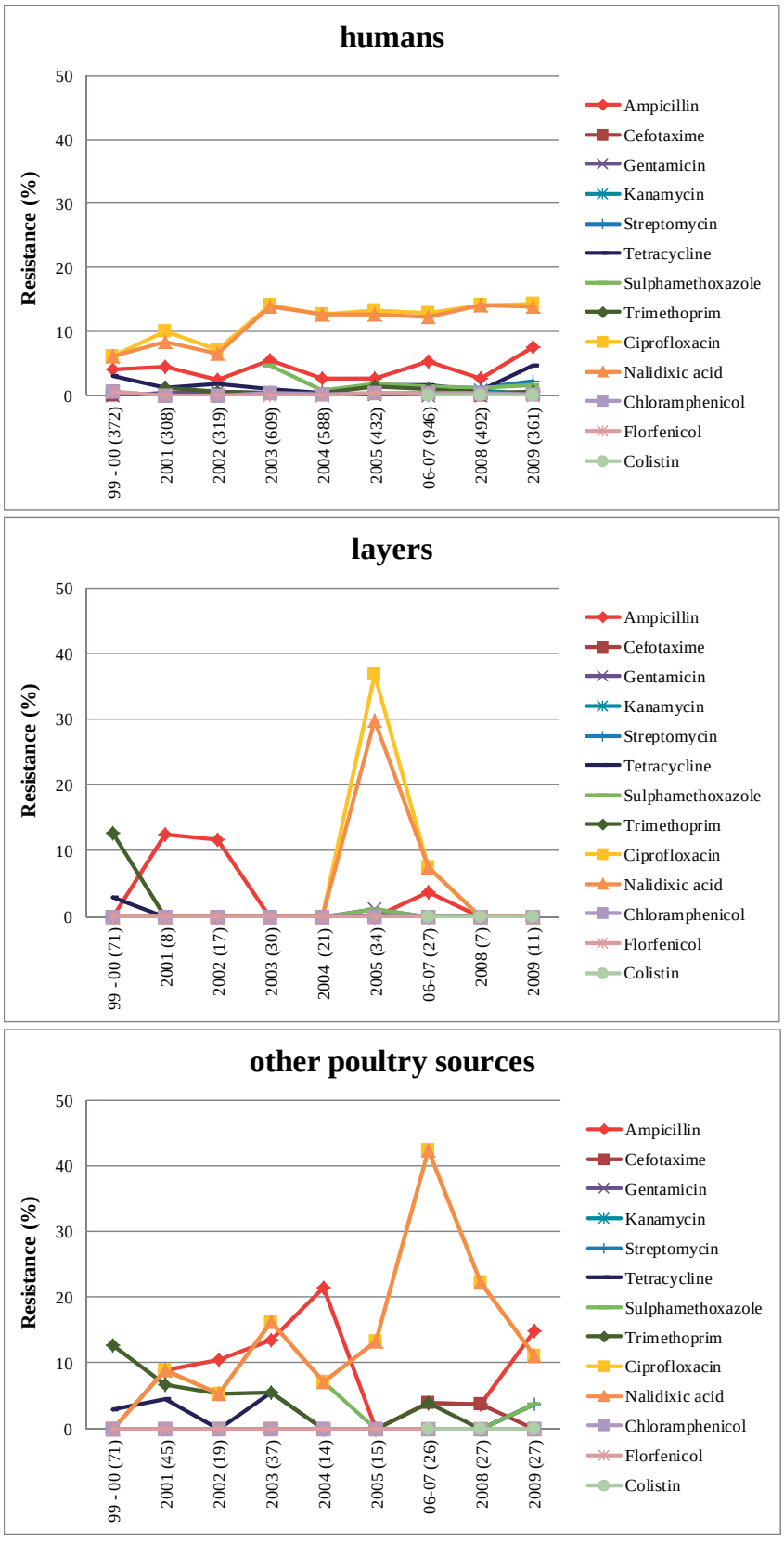


Figure 6.3. Trends in resistance (%) of *S.* Enteritidis isolated from humans, layers and other poultry sources from 1999 – 2009.



S. Typhimurium

As shown in Table 6.1, *S.* Typhimurium represented 32.6% of all human *Salmonella* isolates as characterized by the RIVM in 2009, similar to 2007/2008 (31.3%). *S.* Typhimurium was somewhat less frequently found among animal isolates, however almost all monophasic *S.* subsp. enterica 4,[5],12:i:- belongs to Typhimurium (EFSA opinion) and strongly increased. Indeed, both antimicrobial resistance profile and animal source corresponds with that for *S.* Typhimurium. In 2009, *S.* Typhimurium represented 15.4% of the porcine (compared to 22.0% in 2007/2008), and 17.4% of the bovine isolates (23.2% in 2007/2008). Note that *S.* Typhimurium was not detected in poultry samples in 2009 (compared to 4.0% in 2007/2008). *S.* subsp. enterica 4,[5],12:i:- was discovered occasionally in broilers.

Most notable differences in *S.* Typhimurium phage types isolated from human samples are a decrease in ft507 (from 9.7% in 2007/2008 to 8.2% in 2009) and DT104 (or ft506, from 8.3% to 5.1%), with the emergence in 2009 of ft353 (2.0%) and ft90 (1.7%) (Table 6.1). Ft353 was also present among 0.4% of porcine and 1.1% of bovine isolates, in contrast to ft90 which was not detected among animal isolates available in 2009.

Table 6.5. Resistance percentages of *S.* Typhimurium isolated from different sources and the most prevalent phage types in 2009, including monofasic *S.* subsp. enterica 4,[5],12:i:-.

	***S.* Typhimurium**			**Phage types and monofasic variant**				
	humans (402)	**cattle (20)**	**pigs (28)**	**ft507 (119)**	**ft506 (84)**	**ft90 (21)**	**ft353 (17)**	**SI 1,4,5,12:i:- (104)**
Ampicillin	54.2	70.0	67.9	44.5	82.1	4.8	100.0	77.9
Cefotaxime	0.5	0	0	0	1.2	0	0	1.0
Ceftazidime	0	0	0	0	0	0	0	1.0
Gentamicin	0.7	0	0	0	0	0	0	2.9
Kanamycin	1.2	0	3.6	0.8	0	0	0	3.8
Streptomycin	47.3	60.0	50.0	29.4	92.9	4.8	5.9	82.7
Tetracycline	52.7	45.0	82.1	34.5	89.3	4.8	5.9	86.5
Sulfamethoxazole	61.9	70.0	60.7	70.6	94.0	4.8	0	85.6
Trimethoprim	20.4	15.0	17.9	45.4	8.3	4.8	0	7.7
Ciprofloxacin	5.0	10.0	3.6	0	21.4	4.8	0	1.9
Nalidixic acid	4.0	10.0	3.6	0	20.2	0	0	1.0
Florfenicol	17.7	15.0	17.9	0	81.0	4.8	0	1.0
Chloramphenicol	18.9	15.0	17.9	0	81.0	4.8	0	4.8
Colistin	0	0	0	0	0	0	0	0

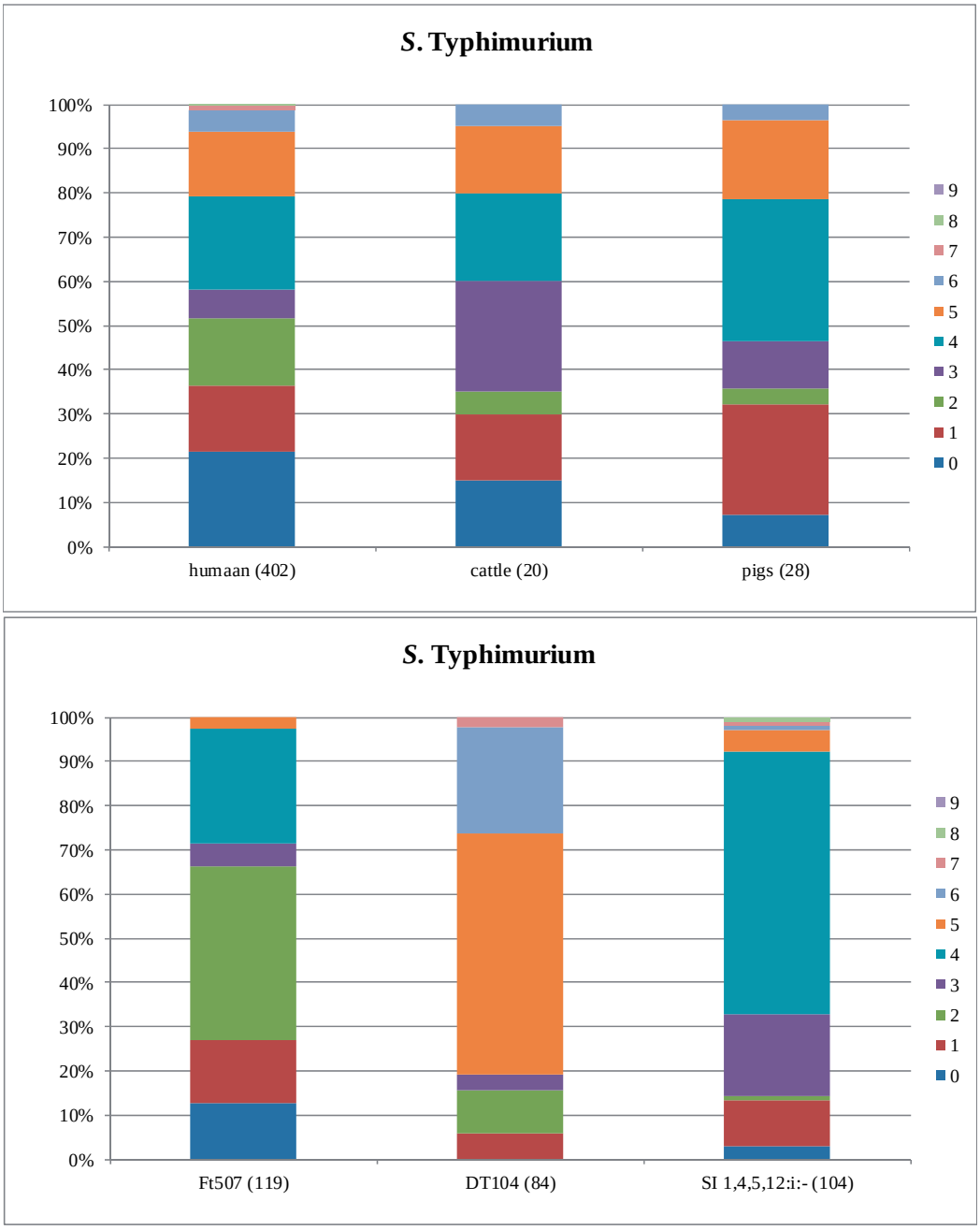
In 2009 the most predominant phage types of *S.* Typhimurium in the collection of strains received from RIVM Bilthoven were: Ft507, Ft506 (≈ DT104), Ft353 and Ft90, of which resistance profiles are shown in Table 6.5. Also the monophasic *S.* subsp. enterica 4,[5],12:i:- is included as this may be regarded as *S.* Typhimurium (EFSA opinion).

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. However, as apparent in Table 6.5, resistance patterns can vary considerably according to Typhimurium phage type.

In 2008, a high prevalence of DT104 was noted, concerning a foodborne outbreak of which the origin was suspected to be abroad. In 2009, the prevalence of this particular phage type decreased considerably, although it was still detected regularly in human, pig and cattle samples (5.1%, 3.1%, and 3.3% of all *Salmonella* isolates respectively).

Multi drug resistance is observed more frequent in *S.* Typhimurium than in *S.* Enteritidis (Figure 6.2 and 6.4). Of the *S.* Typhimurium strains, 48% (humans), 65% (cattle), and 64% (pigs) were resistant to three or more antibiotic classes (Fig. 6.4).

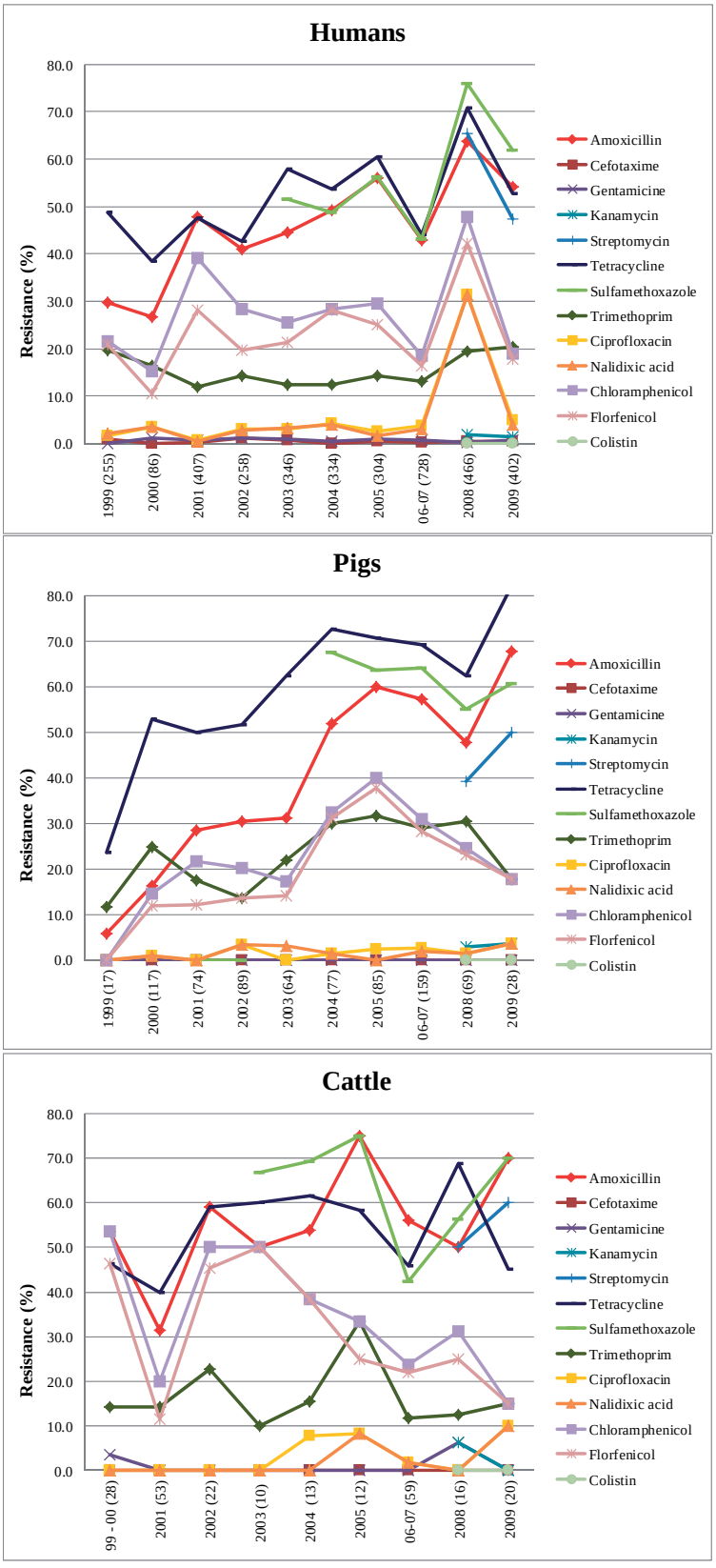
Figure 6.4. Percentages of *S.* Typhimurium strains fully susceptible, resistant to one to nine different antibiotic classes in human and animal sources in the Netherlands in 2009, presented by source (top figure) or phage type, including monofasic *S.* subsp. enterica 4,[5],12:i:- (lower figure).



Resistance in *S.* Typhimurium strains from humans showed a clear increase in 2008 (Fig. 6.5), which was attributed to the increased incidence of quinolone resistant DT104 in human infection in 2008 as a result of a foodborne outbreak. As expected, in 2009 resistance decreased to levels observed in previous years.

With regard to animal strains, resistance levels vary considerably over the years and interpretation should be done with caution because of the relatively small number of the isolates per year. The trend analysis is further affected by the differences in proportion of multi drug resistant phage types per category and per year.

Figure 6.5. Trends in resistance (%) of *S.* Typhimurium isolated from humans and food-animals from 1999 – 2009.

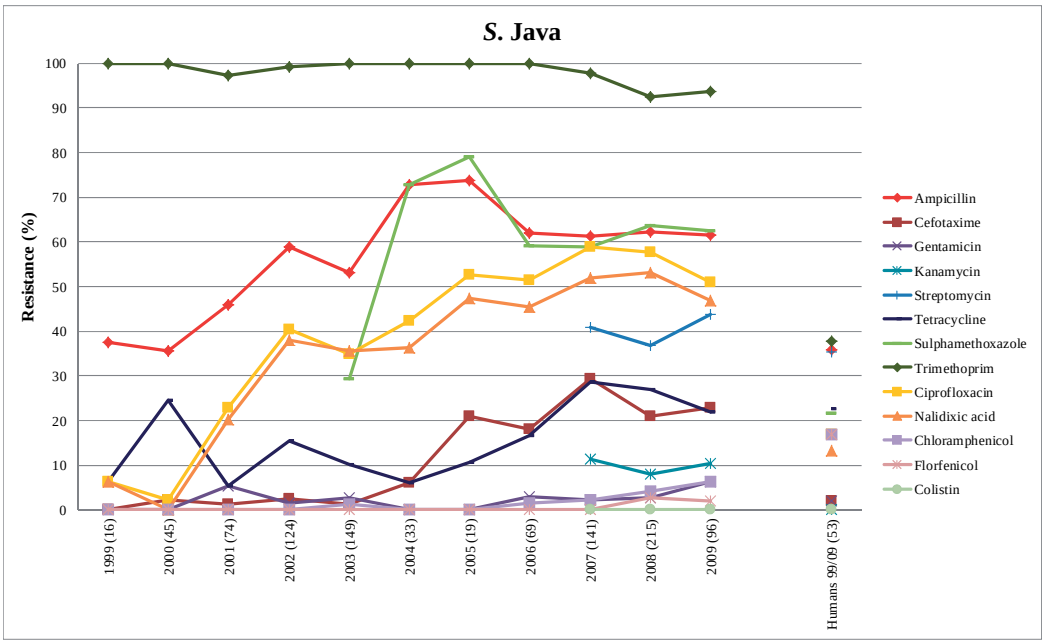


S. Paratyphi B var. Java (S. Java)

As in previous years, in 2009 *S.* Java was the most predominant serovar isolated in broiler production. Roughly half of all *Salmonella* strains isolated from poultry were identified as such.
In 2009, eight *S.* Java strains were isolated from human infections. All but two strains were trimethoprim susceptible and therefore not related to the clone spreading in Dutch poultry and probably travel related. From poultry 77 strains were isolated, all of which harbored the phenotype typical for the clone.
Non-wild type susceptibility to ciprofloxacin occurred in 44 (57%) *S.* Java isolates from poultry, two of which (2.6%) showed high level resistance to ciprofloxacin (MIC 2 mg/L).
Resistance to cefotaxime (ESBL-producers) was detected in 23% of all isolates and 22% of 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 pyramids and was recently described to occur in Canada (Dutil et al., Emerging Infectious Diseases 2010 Jan;16(1):48-54).

In 2009, also in raw poultry meats *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 6.7).

Figure 6.6. Trends in resistance (%) of *S.* Paratyphi B var. Java isolated from poultry from 1999 – 2009 and humans (Separate data on the right indicate all human *S.* Java isolates from 1999 – 2009).



Salmonella in raw meats at retail

Resistance trends are presented for poultry meat only, because in beef and pork the numbers of isolates examined are too small to provide an accurate estimate (Fig. 6.8). The variable contribution of *S.* Java to the annual resistance percentages over all serotypes hampers the interpretation of the observed trend in the resistance. Resistance against cefotaxime and ceftazidime occurred frequently in poultry meat isolates.

Table 6.6. Resistance (%) of *Salmonella enterica* isolated from raw meats from poultry, and other raw meat sources in 2009.

	Poultry *S*. Java N = 53	Poultry other serovars N = 63	Other raw meat sources N = 86
Ampicillin	58.5	12.7	19.8
Cefotaxime	24.5	3.2	0
Ceftazidime	21.2	3.2	0
Gentamicin	7.5	3.2	0
Kanamycin	24.5	9.5	0
Streptomycin	24.5	12.7	19.8
Tetracycline	24.5	23.8	39.5
Sulfamethoxazole	61.2	9.7	53.8
Trimethoprim	98.1	9.5	5.8
Ciprofloxacin	49.1	15.9	1.2
Nalidixic acid	45.3	15.9	0
Chloramphenicol	3.8	1.6	8.1
Florfenicol	0	0	5.8
Colistin	1.9	9.5	0

Figure 6.7. Trends in resistance (%) of *Salmonella enterica* subspecies *enterica* isolated from poultry meats in the Netherlands from 2001 – 2009.

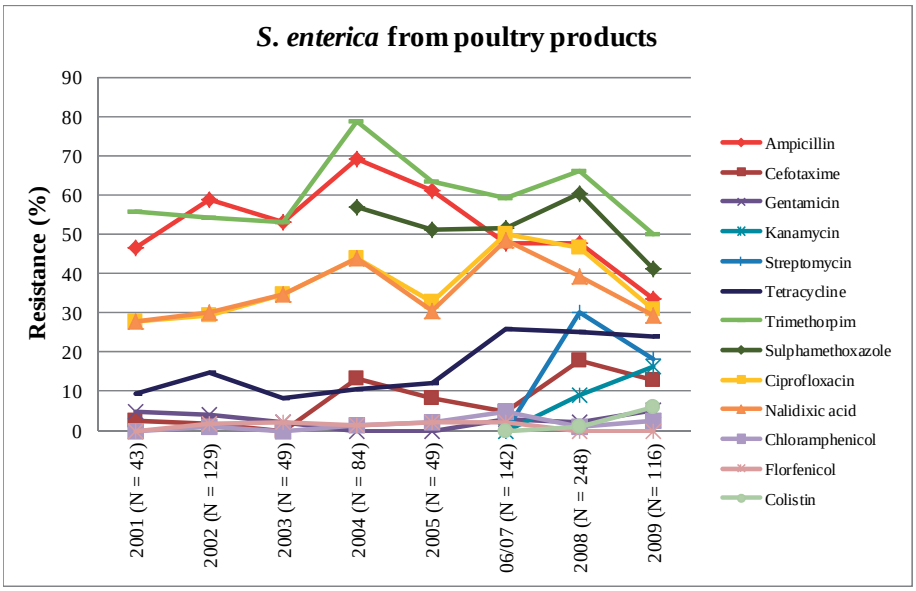


Table 6.7. Distribution of *Salmonella* serovars, in poultry meat at retail (Surveillance data of the New Food and Consumer Product Safety Authority (nVWA) from 1997 – 2009

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
N samples	1,314	1,077	859	1,454	1,578	1,600	1,510	1,482	1,474	1,539	1,403	1,505	1,042
% *Salmonella* spp.	29.1	20.2	17.6	21	16.3	13.4	11.3	7.4	9.4	8.4	8.1	8.1	7.9
(organic)							3.4	2.1	1.9	4			
Paratyphi B Java	15.0	11.4	13.9	33.1	43.2	53.5	45.6	58.2	46.8	38.5	59.6	76.2	62.2
Enteritidis	20.2	12.8	26	6.6	8.2	2.3	8.8	5.5	7.2	6.6	2.0	1,6	17.1
Hadar	10.1	6.1	5	3.3	4.2	0.9	1.8		1.4	5.7	1,0	2.5	2.4
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	2.4
Infantis	9.2	5.0	3.6	6.6	7.0	7.9	11.7		11.5	13.9	13.1	4.9	9.8
Virchow	4.6	2.8	3	10.2	3.5	5.6	5.8	4.5	8.6	11.5	4	1.6	
Typhimurium	7.8	3.6	1.3	0.1	7.4	7.4	5.8	3.6	5.0	1.6	1.0	0.8	0
(DT104)	0	1.8	0.7	0.1	7.0	2.8	5.3	0	2.2	0			
Corvallis									4.3	1.6			
other	27.0	53.6	39.7	30.0	22.3	23.3	19.9	26.4	13.0	16.5	13.2	11.6	6.1

Distribution of Salmonella serovars, in poultry meat.

Table 6.7 presents the contamination rates of *Salmonella* in poultry meat products over the years. The contamination decreased from 29.1% in 1997 to a stable rate of approximately 8% in the last few years.
The distribution of *Salmonella* serovars in poultry meat correspond with those from fecal samples (Tables 6.1 and 6.7), the contamination rate of *S.* Java continues to predominate while for the first year, *S.* Typhimurium was not identified. Also for *S.* Enteritidis, after a decrease to low levels in the previous years the contamination rate in meat shows a remarkable increase, like in live poultry (Table 6.1).

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

Table 6.8 presents the most prevalent serovars found in animal feeds from 2001 – 2009 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 again 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 incidentally present in isolates from soy and compound feeds.
In *Salmonella* isolates from turkeys and horses, more resistance was observed than in strains from ducks, 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 – 2009. Additionally, R% of *Salmonella* strains isolated from incidental animal sources over 2001 – 2009 are presented.

			Animal feed (or ground substance)								Animals				
			Fish meal (59)	Animal meal (72)	Soy (feed, N=1049)	Rapeseed (feed, N=372)	Single feed, other (403)	Composite feed (162)	Feed 2008-2009, N=225	Feed 2001-2007, N=1892	Turkey (104)	Horse (47)	Duck (20)	Pigeon (45)	Reptilian/Amfibian (69)
serovar	**N**	**Antibiotics**	**% resistant isolates 2001-2009**						**% R**	**% R**	**% resistent 2001-2009**				
Senftenberg	269	**amoxicillin**	0	1.4	0.5	0.8	1.5	2.5	1.3	0.8	43.3	21.3	20	11.1	1.4
Agona	210	**cefotaxime**	0	0	0.2	0	0	0.6	0	0.2	1.9	0	0	0	0
Mbandaka	190	**cefuroxime**	0	0	0	0	0	0	--	0	0	0	--	0	0
Lexington	173	**ceftaziclime**	0	0	0.2	0	0	0	0	0	2.6	0	0	0	--
Rissen	138	**imipenem**	0	0	0	0	0	0	--	0	0	0	--	0	0
Cubana	131	**gentamicin**	0	0	0	0	0.5	0	0.4	0.1	12.5	0	0	0	0
Livingstone	107	**neomycin**	0	1.4	0.5	0	1.7	0	7.8	0	14.9	0	0	0	0
Tennessee	85	**doxy/tetracycline**	0	2.8	0.9	0.3	1.7	5.6	2.7	1.3	42.3	19.1	5	6.7	2.9
Anatum	84	**cotrimoxazol**	0	0	0.5	0	0.8	0	--	0.4	0	33.3	--	0	0
Havana	68	**sulfamethoxazole**	0	0	0.5	1.9	1.8	6	1.8	1.3	35.2	13.8	0	9.1	--
Kentucky	59	**trimethoprim**	0	1.4	0.5	0.8	0.2	3.7	1.3	0.7	11.5	19.1	5	0	0
Oranienburg	45	**ciprofloxacin**	1.7	1.4	0.4	0	1.2	3.7	2.2	0.6	47.1	2.1	15	0	0
Minnesota	39	**naladixic acid**	0	1.4	0.3	0	1	3.1	2.2	0.4	40.4	2.1	10	0	0
Infantis	38	**chloramphenicol**	0	1.4	0.7	0.8	1.7	2.5	0.9	1.1	5.8	17	5	11.1	1.4
Montevideo	38	**florfenicol**	0	0	0.5	0.8	0.7	0	0.4	0.5	3.8	6.4	0	11.1	1.4
Cerro	34	**streptomycin**	--	--	8.3	4	11.5	3.4	8.9	5.6	--	--	--	--	--
Yoruba	31	**kanamycine**	--	--	9.2	12	12.8	3.4	9.8	11.1	--	--	--	--	--
17 main serotypes	1739 (82%)														
all serotypes	2117														

Salmonella in other European countries[7]

Salmonella isolates from humans

Overall, *S.* Enteritidis (57.8%) and *S.* Typhimurium (25.7%) were the two most commonly reported *Salmonella* serovars of all reported confirmed human cases in 2009 in reporting member states. Compared to European figures their relative prevalence differed somewhat in the Dutch situation, in which *S.* Enteritidis and *S.* Typhimurium represented 33.0% and 32.6% of all human cases respectively.

Like in the Netherlands, also in some other European countries *S.* Typhimurium DT104 appears to be declining and monophasic *S.* Typhimurium strains, in particular 4,5,12:i:- is now emerging as a dominant *Salmonella* serovar.

Resistance levels showed considerable variation among different member states, and resistance percentages of Dutch *Salmonella* isolates showed no exceptional differences. The relative high ciprofloxacin resistance reported in *S.* Typhimurium and *S.* Enteritidis in the Netherlands may be the result of frequent usage of quinolones in broiler chickens. Similar to the Netherlands, cefotaxime resistance was overall low in reporting member states.

Salmonella isolates from animals and food

In *S.* Enteritidis from poultry, the overall occurrence of ciprofloxacin resistance was 17 %, with varying levels from 0.4 % to 65% amongst different member states. The levels reported by the Netherlands were modest (6%) compared to the high levels reported by Spain, Portugal and Poland (65%, 48% and 29% respectively). Resistance to cefotaxime in *S.* Enteritidis was 0.2% on average in the reporting member states including the Netherlands.

Resistance in *S.* Typhimurium from pigs and cattle ranged considerably among the different countries. The occurrence of resistance in the Netherlands was not exceptional compared to other European countries. The resistance levels to the clinically important fluoroquinolones and third-generation cephalosporins were comparable to the overall occurrence of resistance in all reporting member states.

[7] The Community Summary Report. Antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2009.

Campylobacter spp.

In Table 6.9 the MIC-distributions and resistance percentages are summarized for all *Campylobacter jejuni* and *Campylobacter coli* strains isolated from broilers, pigs, cattle and veal calves in 2009. Table 6.10 shows the more detailed resistance profiles of *C. jejuni* and *C. coli* according to the different sources (meat as well as from fecal samples from different animal species). Figure 6.7 presents trends over the last decade in resistance of *C. jejuni* and *C. coli* recovered from the different sampling categories.
National surveillance data from 2002 onwards for *Campylobacter* spp. isolated from humans are shown in Figure 6.8, and Tables 6.11 and 6.12. Finally, isolation rates of *Campylobacter* in poultry products at retail from 1998-2009 are depicted in Figure 6.9.

Highlights

In *C. jejuni* in animal species highest levels of resistance were detected against tetracycline (45.4%), ampicillin (40.2%) and the quinolones (37.1% and 38.1% for ciprofloxacin and nalidixic acid, respectively). Resistance against tetracyclines varied among different animal species, with very high levels in veal calves (70% for *C. jejuni* and 96.8% for *C. coli*). In *C. jejuni* from human origin, highest level of resistance was noted against fluoroquinolones (53.2%), followed by tetracycline (26.5%). In 2009, 1.3% of the isolates were resistant against erythromycin.
Resistance to macrolide antibiotics (erythromycin, clarithromycin and tulathromycin) was more commonly observed in *C. coli* (17.7%) than in *C. jejuni* (2.1%). Notable is the high level of resistance in *C. coli* from veal calves, with 25.8% of the isolates resistant against erythromycin, clarithromycin and tulathromycin.
In 2009 a decrease in quinolone resistance was observed in *Campylobacter* from broilers and poultry meat, after a steady increase in previous years. Among *C. coli* from pigs, the low level of resistance against the quinolones (5.1%) is remarkable and consistent with previous years.

In 2009, among *C. jejuni* highest resistance levels were observed for ampicillin (40.2%), tetracycline (45.5%) and the quinolones nalidixic acid (38.1%) and ciprofloxacin (37.1%). However, in general, resistance was more frequent in *C. coli*, where additional resistance was commonly observed against streptomycin (in 70.8% of the isolates), and sulfamethoxazole (50.8%). Also with regard to macrolide drugs (erythromycin, tulathromycin ad clarithromycin) resistance was more frequently present in *C. coli* (17.7%) compared to *C. jejuni* (2.1%).

Quinolones

For the first time in years, 2009 saw a decrease in resistance against nalidixic acid and ciprofloxacin in *C. jejuni* isolated from poultry. Ciprofloxacin resistance in *C. jejuni* from broilers and poultry meat was observed in 42.6% and 48.1% of the isolates respectively (Table 6.10.). In contrast, ciprofloxacin resistance among *C. jejuni* recovered from humans continued to increase from 50.5% in 2008 to 53.1% in 2009 as shown in Figure 6.8. and Table 6.11.
Also in *C. coli* from broiler chickens, the ciprofloxacin resistance rate was lower in 2009 compared to the previous year (from 86.7% to 62.5%). It should be noted though that the number of strains available for testing were limited. Resistance in *C. coli* from pigs and veal calves for nalidixic acid and ciprofloxacin remained stable at 5.1% and 74.2%, respectively in 2009.

Macrolides

With regard to the macrolide drugs, resistance among *C. jejuni* is still rare in all animal species. In 2009, resistance rates for most sample categories were slightly lower than in the previous year. For instance erythromycin in *C. jejuni* from broiler chickens decreased from 5.6% in 2008 to 1.6% in 2009, while rates in *C. jejuni* from poultry meat were 4.2% in 2008 and 1.3% in 2009. However, these changes are not statistically significant.
Macrolide resistance is more frequently present in *C. coli* compared to *C. jejuni* with similar resistance rates for all three macrolide agents included in the test panel. In 2009, 17.7% of *C. coli* isolates were resistant against erythromycin, tulathromycin as well as against clarithromycin and 2.1% of *C. jejuni* isolates (Table 6.9). Highest level resistance was observed in *C. coli* recovered from veal calves and pigs, probably reflecting the use of macrolides in the pig and veal calf husbandry in the Netherlands (Table 6.10.).
In *Campylobacter* spp. isolated from humans resistance against erythromycin remained stable at a low frequency (Figure 6.8 and Table 6.11).

Table 6.9. MIC distribution (in %) for all *Campylobacter jejuni* (N = 97, of which 61 from broilers, 11 from dairy cows, 5 from pigs and 20 from veal calves) and *Campylobacter coli* (N = 130, of which 79 from pigs, 16 from broilers, 4 from dairy cows and 31 from veal calves)

C. jejuni	MIC (%) distribution mg/L																
(N = 97)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%	95% CI
Ampicillin		1.0			2.1	26.8	29.9	16.5	1.0	22.7						40.2	30.9-50.5
Gentamicin		79.4	17.5	3.1												0	0-0.04
Neomycin			90.7	7.2			1.0			1.0						2.1	0-5.2
Streptomycin				96.9	1.0			1.0		1.0						2.1	0-5.2
Tetracycline			37.1	1.0	16.5	4.1	1.0		1.0	15.5	23.7					45.4	35.1-55.7
Sulfamethoxazole							9.3	22.7	26.8	28.9	9.3			2.1	1.0	3.1	0-7.2
Ciprofloxacin	40.2	20.6	1.0	1.0		1.0	19.6	10.3	6.2							37.1	27.8-46.4
Nalidixic acid					5.2	34.0	21.6	1.0	1.0	1.0	21.6	14.4				38.1	28.9-47.4
Erythromycin			25.8	48.5	19.6	4.1					2.1					2.1	0-5.2
Clarithromycin			19.6	39.2	32.0	5.2	2.1				2.1					2.1	0-5.2
Tulathromycin			50.5	41.2	5.2	1.0					2.1					2.1	0-5.2
Chloramphenicol					42.3	37.1	17.5	3.1								0	0-0.04
C. coli	MIC (%) distribution mg/L																
(N = 130)	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%	95% CI
Ampicillin		0.8			3.1	9.2	26.9	31.5	14.6	13.8						28.5	20.8-36.2
Gentamicin		23.8	64.6	10.8						0.8						0.8	0-2.3
Neomycin			56.2	30.8	0.8	0.8	3.8		0.8	1.5	5.4					12.3	6.9-18.5
Streptomycin				26.2	3.1		0.8	20.8	30.8	3.1	0.8	14.6				70.8	63.1-78.5
Tetracycline			6.2	2.3	2.3	8.5	0.8		1.5	6.9	71.5					89.2	83.8-94.6
Sulfamethoxazole							7.7	14.6	16.9	9.2	0.8		16.9	20.8	13.1	50.8	42.3-59.2
Ciprofloxacin	41.5	29.2	0.8			0.8	7.7	17.7	2.3							28.5	20.8-36.2
Nalidixic acid						15.4	46.9	5.4	3.8		12.3	16.2				28.5	20.8-36.2
Erythromycin			7.7	16.9	31.5	19.2	6.9				17.7					17.7	11.5-24.6
Clarithromycin			7.7	13.8	33.1	17.7	9.2	0.8		0.8	16.9					17.7	11.5-24.6
Tulathromycin			36.2	33.1	10.8	1.5	0.8			2.3	15.4					17.7	11.5-24.6
Chloramphenicol					3.1	30.8	49.2	16.2	0.8							1	0-2.3

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.

Tetracycline

In *Campylobacter* spp. isolated from humans resistance against tetracyclines varied around 20% (Figure 6.8 and Table 6.11), although some discrepancy was seen in data obtained from individual regional Public Health Laboratories in comparison to the national surveillance data (Figure 6.8), which may be the result of different methodologies and interpretive criteria used. For *C. coli* and *C. jejuni* from animal sources, resistance levels against tetracycline were much higher (up to 96.8% in *C. coli* from veal calves) as shown in Table 6.10.

Ampicillin

Compared to 2008, resistance against ampicillin among *Campylobacter* increased significantly (from 27.8% to 40.2% for *C. jejuni* and from 17.3% to 28.5% in *C. coli*). The increase in ampicillin resistance was apparent in all animal species, as well as in samples from meat. The most striking increase was seen in *C. jejuni* and *C. coli* from cattle. For instance ampicillin resistance increased from 0% in dairy cows in 2008 to 27.3% in 2009. Also ampicillin resistance in *C. coli* from veal calves showed a statistical significant increase, from 13.5% in 2008 (n=52) to 35.5% (n=31) in 2009. For veal calves an increase was noted in *C. jejuni* from 9.1% in 2008 to 25.0% in 2009.

Ampicillin resistance in *C. jejuni* and *C. coli* varies considerably in time. In 2008, for *C. coli* from pigs a large increase in resistance was reported against ampicillin, while resistance in *C. jejuni* from broilers showed a marked decrease. However for both combinations, resistance rates in 2009 were again comparable to those seen in previous years (Figure 6.7.).

Table 6.10. Resistance percentages of *Campylobacter jejuni* and *Campylobacter coli* isolated from raw meat from poultry and from faecal samples of broilers, veal calves, dairy cows (only *C. jejuni*) and pigs (only *C. coli*) in 2009.

	C. jejuni				*C. coli*			
	poultry products	broilers	veal calves	dairy cows	poultry products	broilers	veal calves	pigs
N	233	61	20	11	64	16	31	79
Ampicillin	51.9	45.9	25.0	27.3	25.0	31.3	35.5	24.1
Gentamicin	0.4	0	0	0	0	0	3.2	0
Neomycin	3.4	1.6	5.0	0	10.9	0	32.3	7.6
Streptomycin	2.6	1.6	5.0	0	7.8	25.0	58.1	86.1
Tetracycline	53.2	44.3	70.0	27.3	68.8	81.3	96.8	89.9
Sulfamethoxazole	8.6	4.9	0	0	20.3	43.8	22.6	64.6
Ciprofloxacin	48.1	42.6	30.0	27.3	57.8	62.5	74.2	5.1
Nalidixic acid	49.8	44.3	30.0	27	59.4	62.5	74.2	5.1
Erythromycin	1.3	1.6	5.0	0	20.3	6.3	25.8	17.7
Tulathromycin	1.3	1.6	5.0	0	18.8	6.3	25.8	17.7
Clarithromycin	1.3	1.6	5.0	0	17.2	6.3	25.8	17.7
Chloramphenicol	0.4	0	0	0	0	0	3.2	0

For most antimicrobial drugs, resistance rates varied depending on the animal host and *Campylobacter* subspecies. Most striking differences were found among *C. coli* with regard to aminoglycosides, macrolides and quinolones (Table 6.10.). Resistance data in various animal species reflect the application of antibiotics of choice in different animal sectors (Table 6.10). Quinolone resistance is most common among *C. coli* from broiler chickens (62.5%) and veal calves (74.2%), but low in *C. coli* from pigs (5.1%), reflecting the use of this class of antibiotics.

In *Campylobacter* from humans resistance against ciprofloxacin shows a constant tendency to increase, while resistance against tetracycline and erythromycin remain stable. Possible discrepancies in resistances levels observed in humans versus animal isolates may be explained by the fact that mostly disk diffusion is applied at the regional Public Health Laboratories, which is not standardized and for which no accepted interpretive criteria are defined

For *Campylobacter* from poultry, resistance profiles for isolates recovered from faecal samples as well as from meat samples are shown (Table 6.10 and Figure 6.7.). No statistically significant differences were found for resistance rates in *Campylobacter* isolates from the different sources.

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Figure 6.7. Trends in resistance (%) of *Campylobacter jejuni* (isolated from broilers) and *Campylobacter coli* (from broilers and slaughter pigs) from 2000 - 2009 in the Netherlands.

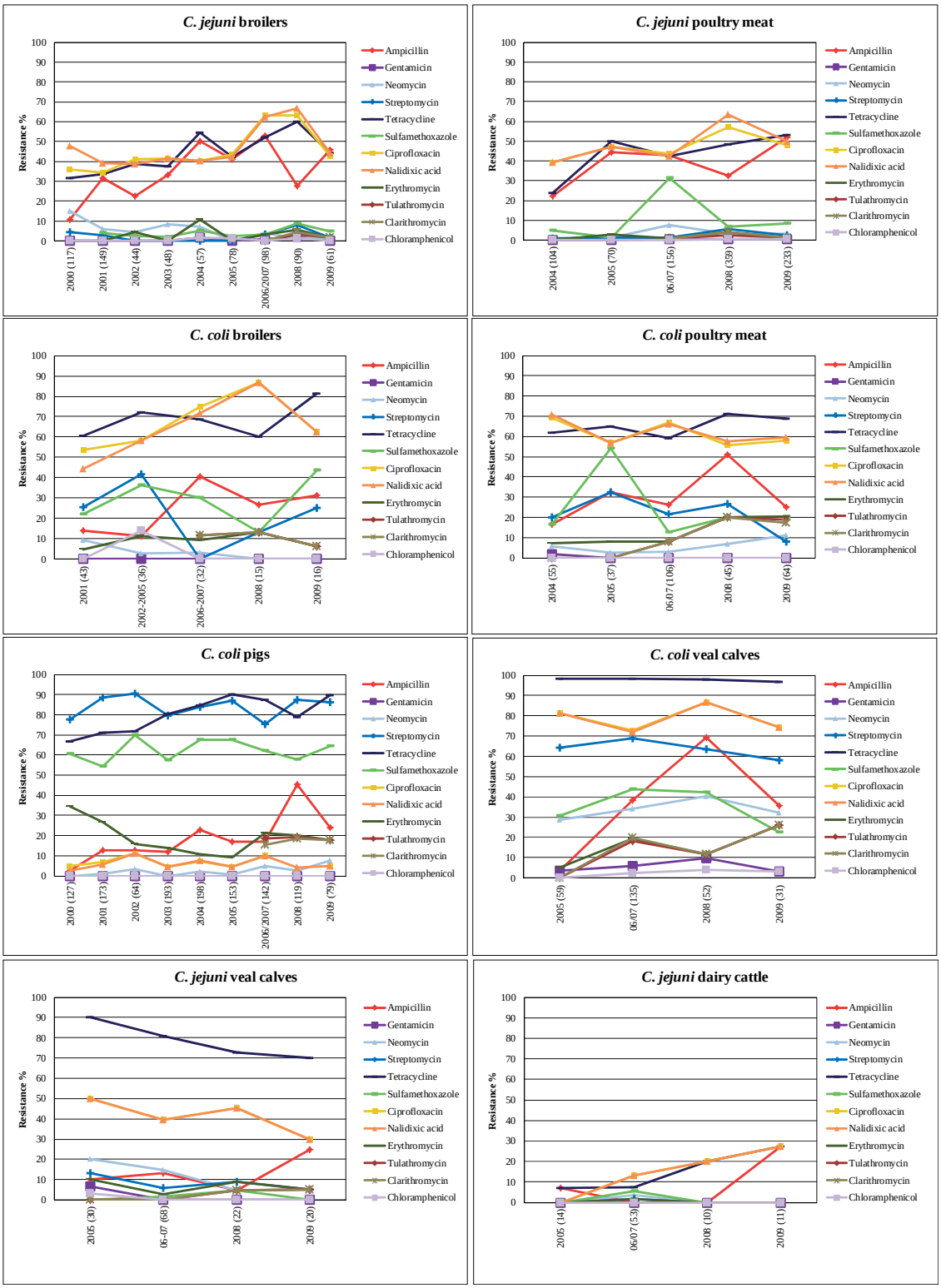


Figure 6.8. Trends in resistance (%) of *Campylobacter* spp. isolated from humans between 1992 and 2009 at the regional Public Health Laboratories (PHLS) of Arnhem and Heerlen covering 990.000 inhabitants (400-700 isolates per year). The continuous line represents national surveillance data from 2002 onwards; the average number of strains tested per year was approximately 2400, ranging from 1900 – 2900.

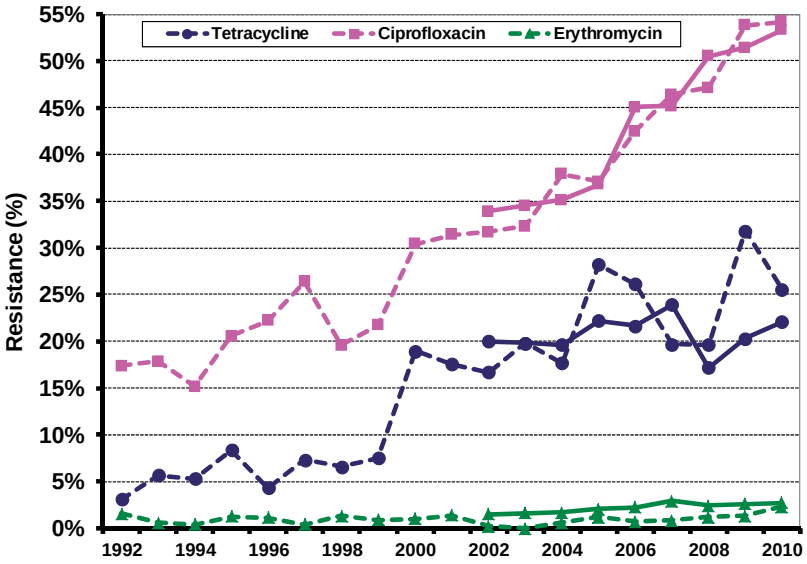


Table 6.11. Percentage *Campylobacter jejuni* and *Campylobacter coli* isolates from humans resistant against fluoroquinolones, tetracycline and erythromycin from 2002 to 2009.

	percentage of resistant *Campylobacter* isolates (%)				
	2002/5	2006	2007	2008	2009
Fluoroquinolone	35.2	45	45.2	50.5	53.1
Tetracycline	20.2	21.7	23.9	17.2	26.5
Erythromycin	1.5	2.2	2.9	2.3	1.3

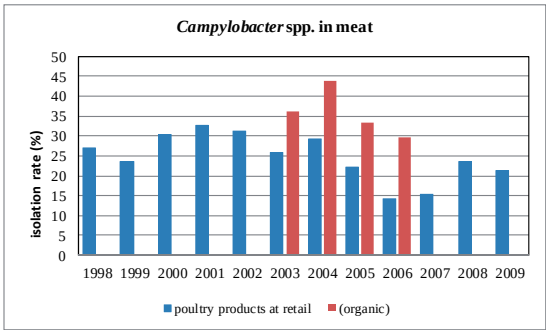
Table 6.12. Domestically acquired and travel related resistance in *C. jejuni* and *C. coli* isolated from humans from 2002 - 2009 from all 16 PHLS covering >50% of the Dutch population.

	2002-2005								2007-2009							
	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**	7091	**48.2**	573	**48.2**	331	**61.9**	31	**54.8**
Tetracycline	5028	**18.5**	353	**22.7**	425	**27.1**	49	**20.4**	4548	**18.6**	437	**31.4**	112	**27.7**	18	**33.3**
Erythromycin	5735	**1.2**	372	**3**	511	**1.6**	52	**0**	5822	**2**	492	**5.5**	256	**3.9**	24	**4.2**

In analogy to previous years, in travel-related Campylobacter infections fluoroquinolone resistance occurred more frequently than in domestically acquired infections as shown in Table 6.12. For tetracycline and erythromycin this difference was observed for *C. jejuni* only.

In the surveillance program carried out by the Dutch new Food and Consumer Product Safety Authority (nVWA), meat from different animal species (sample size 1100-1500 per year) are tested for the presence of zoonotic food pathogens. Isolation rate data for *Campylobacter* spp. are shown in Figure 6.9. After a decline in 2006 and 2007, contamination levels in poultry products are again comparable to the levels in previous years. The levels in organic poultry are slightly higher than in conventionally raised animals.

Figure 6.9. Isolation rates of *Campylobacter* in poultry meat at retail, from 1998-2009.



Campylobacter in other European countries[8]

Antimicrobial resistance levels in *C. jejuni* from humans in the Netherlands were similar (erythromycin and ciprofloxacin) or slightly lower (tetracycline) than the mean of values reported from other European countries.
Also for *Campylobacter* spp. from animals and meat, levels in the Netherlands were generally comparable to the mean of values reported from other European countries. However, the resistance levels in individual countries varied substantially. It should be noted that the guidelines used for the methodology and interpretation of the antimicrobial susceptibility testing for *Campylobacter* may still differ between countries, which complicates the interpretation and comparison of results between countries.

[8] The Community Summary Report. Antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2009

Shiga-toxin producing E. coli O157 (STEC)

Highlights

Among human isolates of STEC *E. coli* O157 resistance levels have been traditionally low. The increase noted in 2008 was absent in 2009. Highest level of resistance among human isolates was noted for streptomycin and sulfamethoxazole (in 9.5% of the isolates).
As expected, resistance in isolates from cattle was more commonly present. Resistance against streptomycin and sulfamethoxazole was seen in 32% and 20% of bovine isolates respectively. Resistance was further noted against tetracycline (17.3%).
Based on MIC profiles, no extended spectrum beta-lactamases (ESBL) suspect phenotypes were present as all human and animal isolates were susceptible to cefotaxime and ceftazidime.

In 2009, 138 Shiga-toxin producing *E. coli* O157 (STEC) isolates were tested for susceptibility. Isolates were obtained from human patients (N = 63) and from cattle (N = 75). MIC results are shown in Table 6.14. Of the bovine isolates, 53 were recovered from veal calves, and 22 from dairy cattle. No statistical significant differences in resistance profiles were noticed between both sample sources (data not shown).

Traditionally, resistance in *E. coli* O157 from human specimens is very low, while in cattle strains resistance occurs more commonly. In 2009, highest levels of resistance in bovine strains were seen against streptomycin (32%) and sulfamethoxazole (20%).

As in previous years, no resistance was detected in human or cattle strains against third generation cephalosporins (cefotaxime or ceftazidime) which is considered to be an indication for the presence of extended spectrum beta-lactamases (ESBLs).

Trends in resistance

Although annual resistance rates vary to some extent, resistance in animal isolates show a tendency to increase over the last few years (Figure 6.9). Also in human isolates, an increase was noted in resistance profiles of 2008. However, in 2009 resistance levels were again fairly low. Highest rates of resistance in strains isolated from human samples were observed for sulfamethoxazole and streptomycin (both 9.5%).

Table 6.14. MIC distribution (in %) for STEC *E. coli* O157 isolated in the Netherlands from human (N=63) and cattle faeces (N=75) in 2009.

Humans	MIC (%) distribution mg/L																			
N = 63	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%	95% CI
Ampicillin								3.2	92.1	1.6			3.2						3.2	0-7.9
Cefotaxime			93.7	6.3															0	0-0.06
Ceftazidime					100.0														0	0-0.06
Gentamicin					3.2	74.6	15.9	4.8	1.6										1.6	0-4.8
Kanamycin									95.2	4.8									0	0-0.06
Streptomycin									54.0	34.9	1.6			3.2	6.3				9.5	3.2-17.5
Tetracycline								47.6	47.6					4.8					4.8	0-11.1
Sulfamethoxazole										90.5								9.5	9.5	3.2-17.5
Trimethoprim						98.4							1.6						1.6	0-4.8
Ciprofloxacin	68.3	31.7																	0	0-0.06
Nalidixic acid									98.4	1.6									0	0-0.06
Chloramphenicol									3.2	69.8	25.4			1.6					1.6	0-4.8
Florfenicol									6.3	77.8	15.9								0	0-0.06
Colistin								98.4	1.6										0	0-0.06

Cattle	MIC (%) distribution mg/L																			
N = 75	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%	95% CI
Ampicillin								16.0	70.7	9.3			4.0						4.0	0-9.3
Cefotaxime			94.7	1.3	4.0														0	0-0.05
Ceftazidime					98.7	1.3													0	0-0.05
Gentamicin					20.0	64.0	12.0		4.0										4.0	0-9.3
Kanamycin									90.7	2.7					6.7				6.7	1.3-13.3
Streptomycin									50.7	16.0	1.3	1.3	20.0	4.0	6.7				32.0	21.3-42.7
Tetracycline								18.7	64.0		1.3	2.7		13.3					17.3	9.3-26.7
Sulfamethoxazole										20.0	30.7	29.3						20.0	20.0	12-29.3
Trimethoprim						90.7	1.3						8.0						8.0	2.7-14.7
Ciprofloxacin	50.7	45.3	2.7	1.3															1.3	0-4
Nalidixic acid									94.7	5.3									0	0-0.05
Chloramphenicol										84.0	8.0			8.0					8.0	2.7-14.7
Florfenicol									25.3	70.7				4.0					4.0	0-9.3
Colistin										100.0									0	0-0.05

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 – 2009.

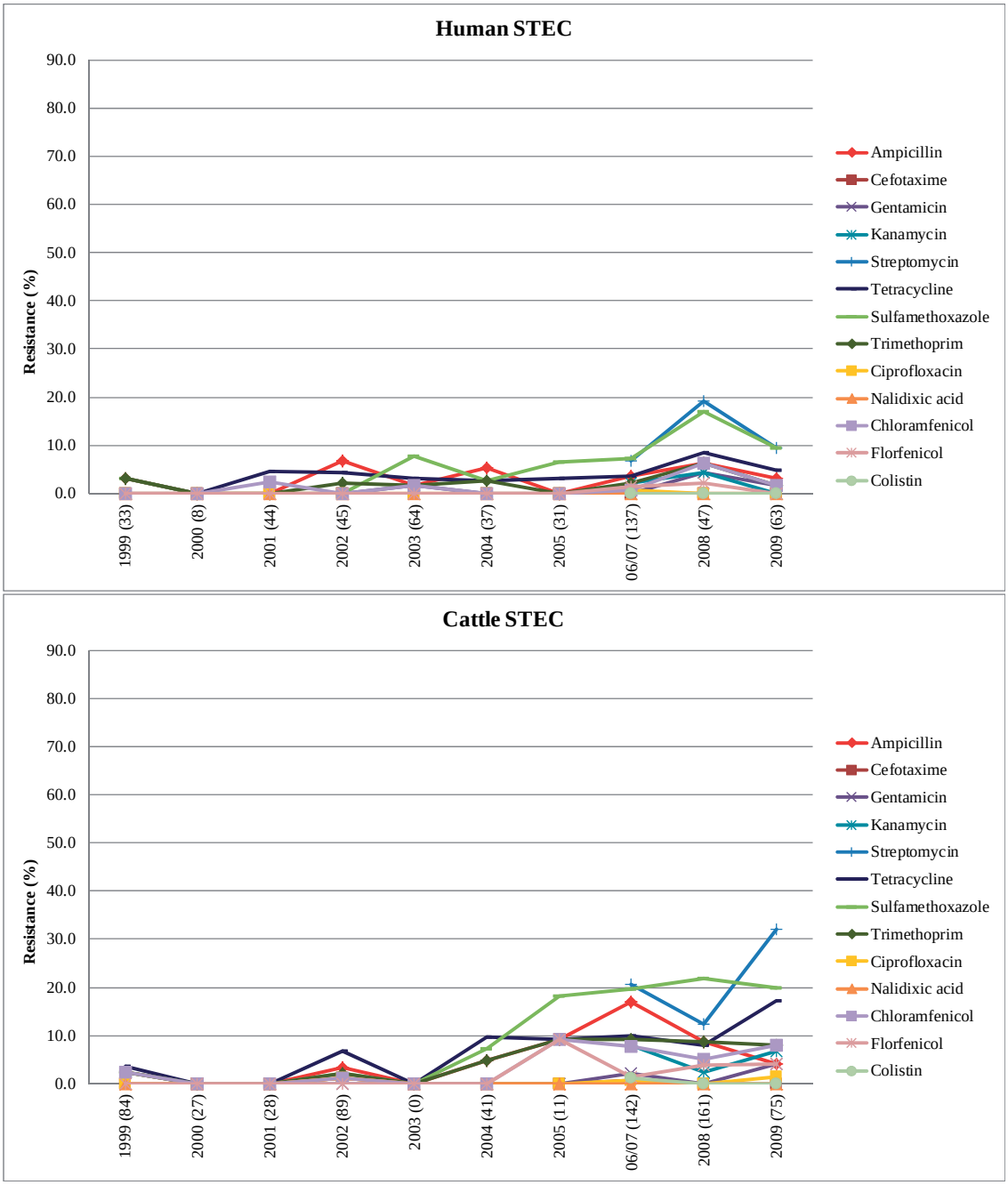
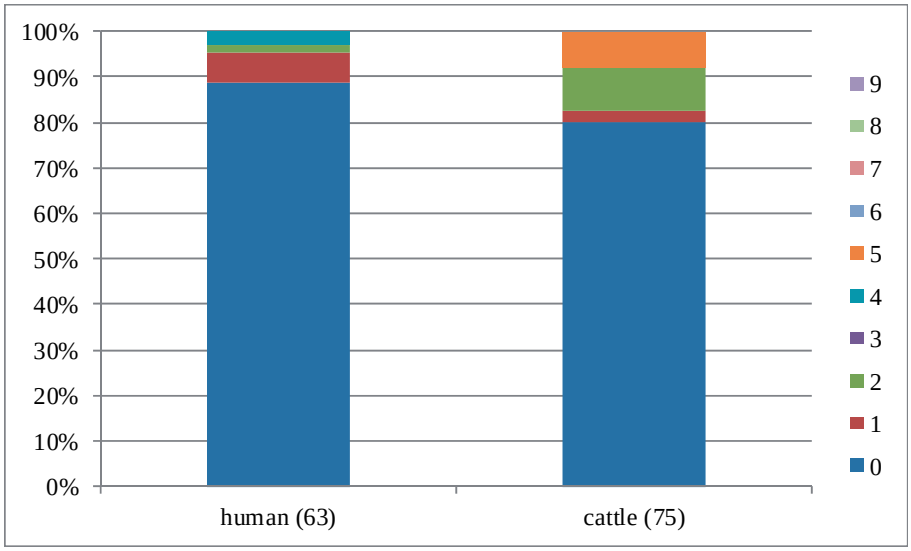


Figure 6.10. Percentages of STEC *E. coli* O157 strains fully susceptible, resistant to one to a maximum of nine antimicrobial classes from human and cattle specimens in 2009.



Multidrug resistance

Information on multiple drug resistance is shown in Figure 6.10. Concerning human isolates, three out of 63 isolates (5%) were resistant against two or more classes of antibiotics. All three isolates were resistant against tetracyclines and sulfamethoxazole, with additional resistance against ampicillin and trimethoprim (one isolate) or ampicillin and chloramphenicol (one isolate). With respect to isolates from cattle, 13 out of 75 isolates (17%) were resistant against two or more classes of antibiotics. Resistance profiles were similar to those from human origin, i.e. all "multidrug resistant" isolates were resistant against tetracyclines and sulfamethoxazole, with additional resistance in six isolates (8%) against trimethoprim, chloramphenicol, aminoglycosides and/or ampicillin.

7. Commensal indicator organisms

This chapter describes the susceptibility profiles of commensal micro-organisms of the gastro-intestinal (GI) tract. The level of antimicrobial resistance in bacteria inhabiting 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* species (*E. faecium* and *E. faecalis*) are included as indicator organisms for the Gram-negative and the Gram-positive flora, respectively.

Isolation of bacteria from the intestine of randomly picked animals (broiler chickens and pigs) 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 have been included in the monitoring, using the same samples that were taken at farms to determine the prevalence of *Salmonella*, *E. coli* O157 and *Campylobacter*.

It should be noted however, that 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 set of selected isolates is intended to represent the *E. coli*, or *Enterococcus* species population of each animal species of the entire country. One per cent 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 10^4 cfu/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

This chapter presents information on resistance in *Escherichia coli* from food-producing animals in the Netherlands as indicator organisms for the occurrence and trends in resistance in Gram-negative bacteria present in the GI-tract of food-producing animals. Isolates were selected from chickens, pigs, and cattle. In 2009, for 1317 *E. coli* strains MIC values have been determined. In addition to susceptibility profiles of 897 isolates from live animals, data on 420 *E. coli* isolated from raw meat products are presented.

In Table 7.1 MIC distributions are summarized for *E. coli* strains isolated from fecal samples in 2009. Figure 7.1 presents information on trends in resistance levels over time according to host animal species. Trends in multidrug resistance are depicted in Figure 7.2.
Data for 2009 on *E. coli* isolated from different meat products are presented in Table 7.2. as well as in Figure 7.3 (showing trends in resistance percentages from 2002 to 2009).

Highlights

For most antibiotic classes the trend for increasing resistance continues in 2009. Notably resistance against beta-lactam antibiotics is disturbing. Resistance against 3rd and 4th generation of cephalosporins, indicative of ESBL producing *E. coli*, has further increased compared to 2008. Cefotaxime resistance was detected in all animal host species, ranging from 1.5% in dairy cattle to 17.9% in broiler chickens.
The increase of resistance in commensal *E. coli* isolated from dairy cows noted in the last five years was again apparent in 2009.

Table 7.1 presents the MIC distributions and resistance percentages for *E. coli* strains isolated from farm animals in 2009 specified for broiler chickens, slaughter pigs, veal calves and dairy cows. Although resistance rates vary among the different animal species, universally highest resistance is seen against ampicillin, tetracycline, streptomycin, trimethoprim and sulfamethoxazole. These include the drug classes that are most frequently used in veterinary medicine.
In addition, reduced susceptibility to quinolones is commonly encountered in *E. coli* isolated from broiler chickens; 57.4% of all isolates that were tested showed non-wild type susceptibility[9] to nalidixic acid and ciprofloxacin. In 2009 high level resistance (MIC >1 mg/L) against ciprofloxacin in broiler chickens was detected in 5.4%, compared to 6.3% of the isolates in 2008.

Poultry

In the last decade the resistance percentages of *E. coli* from broilers show a tendency to increase for most antibiotics included in the test panel. For beta-lactam antibiotics this increase was substantial. In 2009 as much as 73.2% of commensal *E. coli* from broilers were resistant against ampicillin, while 17.9% of the *E. coli* strains were resistant against cefotaxime, indicative of ESBL production (Table 7.1). In comparison, in 1998 resistance rates against ampicillin and cefotaxime were 38.9% and 2.6% respectively.
In broilers tetracycline resistance seems to be fairly stable over the years, varying from 52.6% (2006) to 66.7% (2004). In 2009, tetracycline resistance was observed in 61.9% of the *E. coli* isolated from broiler chickens. Also resistance against trimethoprim and sulfamethoxazole was widespread in commensal *E. coli* from broiler chickens (62.2% and 71.5% respectively).
With respect to aminoglycoside antibiotics, highest resistance rates were seen against streptomycin (67.4%), representing an increase since 2007 (51.2%) and 2006 (60.0%) when streptomycin was first included in the selection of antibiotics tested. Moreover, the majority of resistant *E. coli* showed high level resistance against streptomycin as reflected in MIC ≥256 mg/l. Resistance against kanamycin (17.2%) and gentamicin (8.6%) was less commonly seen.

[9] 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 broiler chickens (N=291), slaughter pigs (N=296), veal calves (N=171) and dairy cattle (N=136) in The Netherlands in 2009.

Broilers	MIC (%) distribution mg/L																			
N = 291	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%	95% CI
Ampicillin							0.7	6.5	16.2	3.4	0.3		72.9						73.2	68-78.4
Cefotaxime			63.2	17.2	1.7		0.7	0.3	1.0	15.8									17.9	13.7-22.3
Ceftazidime					79.4	3.1	3.4	6.5	0.7	1.7	3.1	2.1							17.5	13.4-22
Gentamicin					1.0	43.6	37.1	9.6	1.4	0.7	2.4	2.7	1.4						8.6	5.5-12
Kanamycin									63.2	19.6	5.2				12.0				17.2	13.1-21.6
Streptomycin									5.8	18.9	7.9	6.9	7.9	9.6	43.0				67.4	61.9-72.5
Tetracycline							1.4	16.5	18.9	1.4	0.3	0.3	5.2	56.0					61.9	56.4-67.4
Sulfamethoxazole										28.2				0.3				71.5	71.5	66.3-76.6
Trimethoprim						37.5	0.3						62.2						62.2	56.7-67.7
Ciprofloxacin	34.4	7.6	0.7	6.5	27.8	14.8	2.7		1.0	1.7	2.7								57.4	51.5-62.9
Nalidixic acid									41.9	0.3	0.3	0.7	7.6	49.1					57.4	51.5-62.9
Chloramphenicol									7.2	55.3	13.7	2.1	4.8	16.8					23.7	18.9-28.5
Florfenicol									5.8	69.1	21.0	3.4	0.7						4.1	2.1-6.5
Colistin								0.3		99.7									0	0-0.01

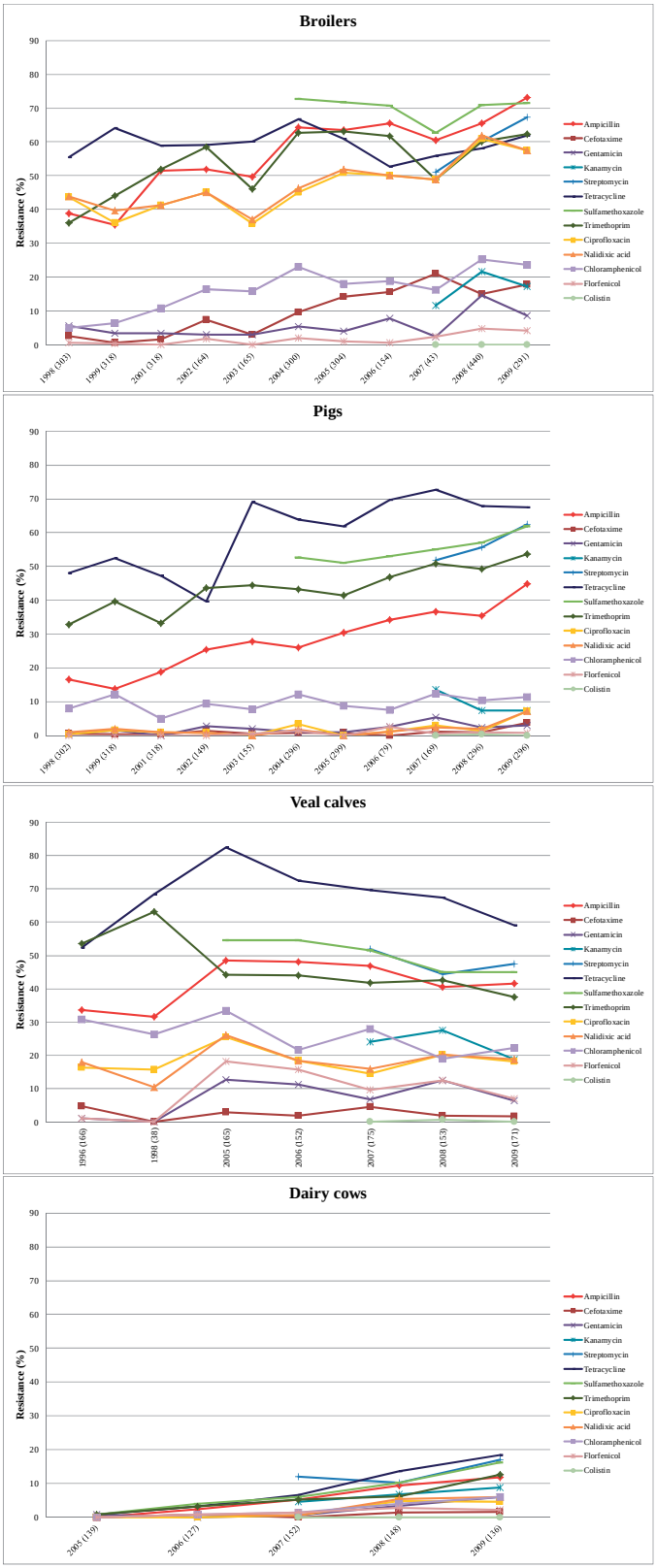
Slaughter pigs	MIC (%) distribution mg/L																			
N = 296	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	R%	95% CI
Ampicillin							2.7	13.5	36.1	2.7		0.3	44.6						44.9	39.2-50.7
Cefotaxime			85.8	10.1	0.3			0.3		3.4									3.7	1.7-6.1
Ceftazidime					92.2	4.1	1.4	1.0		0.3		1.0							3.7	1.7-6.1
Gentamicin					3.0	47.6	37.8	8.4	3.0										3.0	1.4-5.1
Kanamycin									78.7	13.9	3.7	0.3			3.4				7.4	4.7-10.5
Streptomycin									7.4	18.9	11.1	6.8	10.5	13.2	32.1				62.5	57.1-67.9
Tetracycline							2.7	13.2	14.9	1.7	1.0		6.8	59.8					67.6	62.2-73
Sulfamethoxazole										38.2								61.8	61.8	56.4-67.2
Trimethoprim						45.6	0.7						53.7						53.7	48-59.5
Ciprofloxacin	73.0	19.3	0.7	1.4	4.1	1.4					0.3								7.1	4.4-10.1
Nalidixic acid									92.6		0.3		1.0	6.1					7.1	4.4-10.1
Chloramphenicol								0.7	8.8	68.6	10.5	3.0	1.4	7.1					11.5	8.1-15.2
Florfenicol									10.5	70.9	17.9			0.7					0.7	0-1.7
Colistin										100.0									0	0-0.01

Veal calves	MIC (%) distribution mg/L																			
N = 171	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%	95% CI
Ampicillin							0.6	12.3	43.3	2.3			41.5						41.5	33.9-49.1
Cefotaxime			81.3	13.5	3.5					1.8									1.8	0-4.1
Ceftazidime					94.2	3.5	1.2			0.6	0.6								2.3	0.6-4.7
Gentamicin					0.6	42.1	42.7	8.2		0.6	1.8	0.6	3.5						6.4	2.9-10.5
Kanamycin									70.2	11.1	2.3			0.6	15.8				18.7	12.9-24.6
Streptomycin									9.4	33.3	9.9	4.1	5.8	7.0	30.4				47.4	39.8-55
Tetracycline							0.6	11.1	27.5	1.8			4.1	55.0					59.1	51.5-66.1
Sulfamethoxazole										54.4	0.6							45.0	45.0	37.4-52.6
Trimethoprim						62.0	0.6						37.4						37.4	30.4-45
Ciprofloxacin	62.0	19.3	0.6		8.2	2.3	1.2	1.2	0.6		4.7								18.1	12.3-24
Nalidixic acid									80.1	1.2		0.6	2.3	15.8					18.7	12.9-24.6
Chloramphenicol									4.7	52.6	20.5		1.2	21.1					22.2	16.4-28.7
Florfenicol									4.1	64.3	24.6		0.6	6.4					7.0	3.5-11.1
Colistin										100.0									0	0-0.02

Dairy cattle	MIC (%) distribution mg/L																			
N = 136	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%	95% CI
Ampicillin							2.2	23.5	58.8	3.7			11.8						11.8	6.6-17.6
Cefotaxime			75.0	22.8	0.7					1.5									1.5	0-3.7
Ceftazidime					96.3	2.2		1.5											1.5	0-3.7
Gentamicin					2.9	53.7	30.9	6.6	1.5		2.2	0.7	1.5						5.9	2.2-10.3
Kanamycin									79.4	11.8	3.7		0.7		4.4				8.8	4.4-14
Streptomycin									20.6	51.5	11.0	2.2	2.9	2.2	9.6				16.9	11-23.5
Tetracycline							2.2	33.8	43.4	2.2	0.7		1.5	16.2					18.4	11.8-25
Sulfamethoxazole										83.8								16.2	16.2	10.3-22.8
Trimethoprim						85.3	2.2						12.5						12.5	7.4-18.4
Ciprofloxacin	68.7	26.9		0.7	3.0		0.7												4.5	1.5-8.2
Nalidixic acid									92.6	0.7	0.7		0.7	5.1					5.9	2.2-10.3
Chloramphenicol									2.9	73.5	17.6	1.5	2.2	2.2					5.9	2.2-10.3
Florfenicol									2.9	78.7	16.2	0.7		1.5					2.2	0-5.1
Colistin										100.0									0	0-0.03

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 as defined by EUCAST. The dashed bars indicate the clinical breakpoints.

Figure 7.1. Trends in resistance (in%) of *E. coli* isolated from broilers, slaughter pigs, veal calves, and dairy cattle in the Netherlands from 1998 – 2009.



Pigs

Compared to 2008, *E. coli* isolated from slaughter pigs showed an increase in resistance for most antibiotics. Especially with regard to beta-lactam antibiotics (ampicillin, cefotaxime and ceftazidime), fluoroquinolones (nalidixic acid and ciprofloxacin), trimethoprim and sulfamethoxazole. This is an on-going trend since approximately the turn of the century as shown in Figure 7.1. For instance ampicillin resistance raised from 16.6% in 1998 to 44.9% in 2009. The observed 3.7% of the isolates being resistant against cefotaxime and ceftazidime resistance could indicate that similar to what was observed in broilers, also in pigs the prevalence of ESBL-producers is increasing.
With regard to tetracycline, in 1998 resistance was detected in 48.0% of the *E. coli* strains, increasing to 67.6% in 2009. Also for trimethoprim and sulfamethoxazole a significant increase in resistance is seen, from 32.8% (1998) to 53.7% and 52.7 (2004) to 61.8% respectively.
Resistance is variable for the aminoglycoside class antibiotics. Streptomycin resistance is common (62.5% in 2009), while 3.0% of the *E. coli* strains were resistant against gentamicin and 7.4% against kanamycin.
Resistance against chloramphenicol and florfenicol has remained stable over the years, with an average rate around 10% for chloramphenicol and below 1% for florfenicol.

Veal calves

Over the last five years, resistance rates in *E. coli* isolated from veal calves seem to be relatively constant or show a slight tendency to decrease. For beta-lactam antibiotics, resistance against ampicillin is commonly seen (41.5% in 2009). However, resistance against 3rd generation of cephalosporins like cefotaxime and ceftazidime remains relatively low (1.8 and 2.3% respectively).
Remarkably, resistance against tetracyclines has decreased from 2005 onwards. In 2005 as much as 82.4% of all *E. coli* form veal calves tested were resistant against tetracycline, compared to 59.1% in 2009.
With regard to fluoroquinolone resistance, about 18.1% of *E. coli* from veal calves showed reduced susceptibility to ciprofloxacin, 6.5% were considered clinically resistant with MIC values >1 mg/L. The percentage of *E. coli* with acquired resistance mechanisms against ciprofloxacin in 2009 was higher among veal calves compared to pigs (7.1%) and dairy cattle (4.5%), but lower than seen in broilers (57.4%). This likely reflects the use of quinolones in various animal husbandries.

Dairy cattle

In general, resistance in *E. coli* isolated from dairy cattle is low compared to resistance levels seen in pigs, broilers and veal calves. However, in dairy cattle a prominent increase in resistance has been observed over the last five years as shown in Figure 7.1. In 2009, resistance was observed for all antibiotics tested except colistin. Highest resistance was observed for tetracycline (18.4% of *E. coli* isolated from dairy cows), followed by streptomycin (16.9%), and sulfamethoxazole (16.2%). A plausible explanation for this increase in resistance is currently lacking.

Multidrug resistance

The overall increase in resistance is also reflected in de multidrug resistance data as shown in Figure 7.2. In commensal *E. coli* from all animal host species, resistance was discovered against up to eight out of nine tested classes of antibiotics (represented by ampicillin, cefotaxime, gentamicin, tetracycline, sulfamethoxazole, trimethoprim, nalidixic acid, chloramphenicol, and kanamycin).
The highest level of multidrug resistance was present among *E. coli* originating from broilers. Only 12% of the commensal *E. coli* strains from broiler chickens were fully susceptible to all antimicrobials tested. As much as 27.5% of the isolates showed resistance to six or more classes of antibiotics, 2.5% was resistant to eight classes. Also among *E. coli* from veal calves and pigs, multidrug resistance was widespread although less common compared to broilers. Amid *E. coli* from veal calves, 11.1% was resistant to six or more classes of antibiotics, for porcine *E. coli* this was the case for 4.4% of the isolates. For *E. coli* from dairy cattle multidrug resistance is most favourable, although rapidly expanding. In 2005, less than 2% of the *E. coli* from dairy cattle was resistant to one or more antibiotics. In 2009, this increased more than tenfold with 2.9% of *E. coli* strains showing reduced susceptibility to eight antibiotic classes.

Figure 7.2. Trends in percentages of *E. coli* strains fully susceptible, resistant to one to a maximum of nine tested antimicrobial classes in broiler chickens, slaughter pigs and veal calves in the Netherlands from 1998 - 2009.

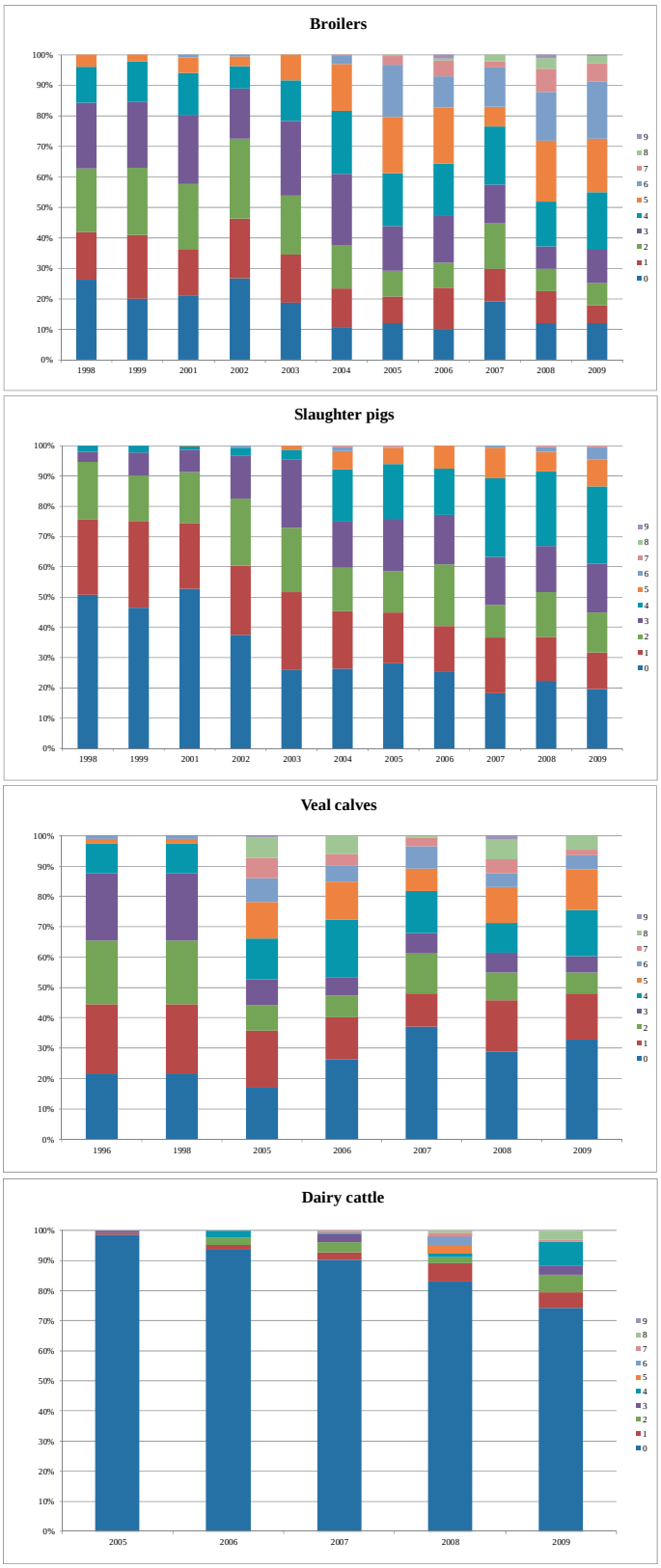


Table 7.2. Resistance (in %) of *E. coli* isolated from raw meat products at retail in the Netherlands in 2009.

	Poultry meat N = 328	Pork N = 13	Beef N = 79
Ampicillin	67.7	30.8	15.2
Cefotaxime	21.3	7.7	1.3
Ceftazidime	18.0	7.7	1.3
Gentamicin	10.4	0.0	2.5
Kanamycin	19.5	15.4	8.9
Streptomycin	55.5	53.8	17.7
Tetracycline	60.1	69.2	17.7
Sulfamethoxazole	90.9	92.3	96.2
Trimethoprim	53.0	46.2	8.9
Ciprofloxacin	49.7	0.0	3.8
Naladixic acid	48.8	0	3.8
Chloramphenicol	22.3	23.1	8.9
Florfenicol	2.4	0	3.8
Colistin	2.1	0.0	0.0

E. coli in raw meat products of food-animals

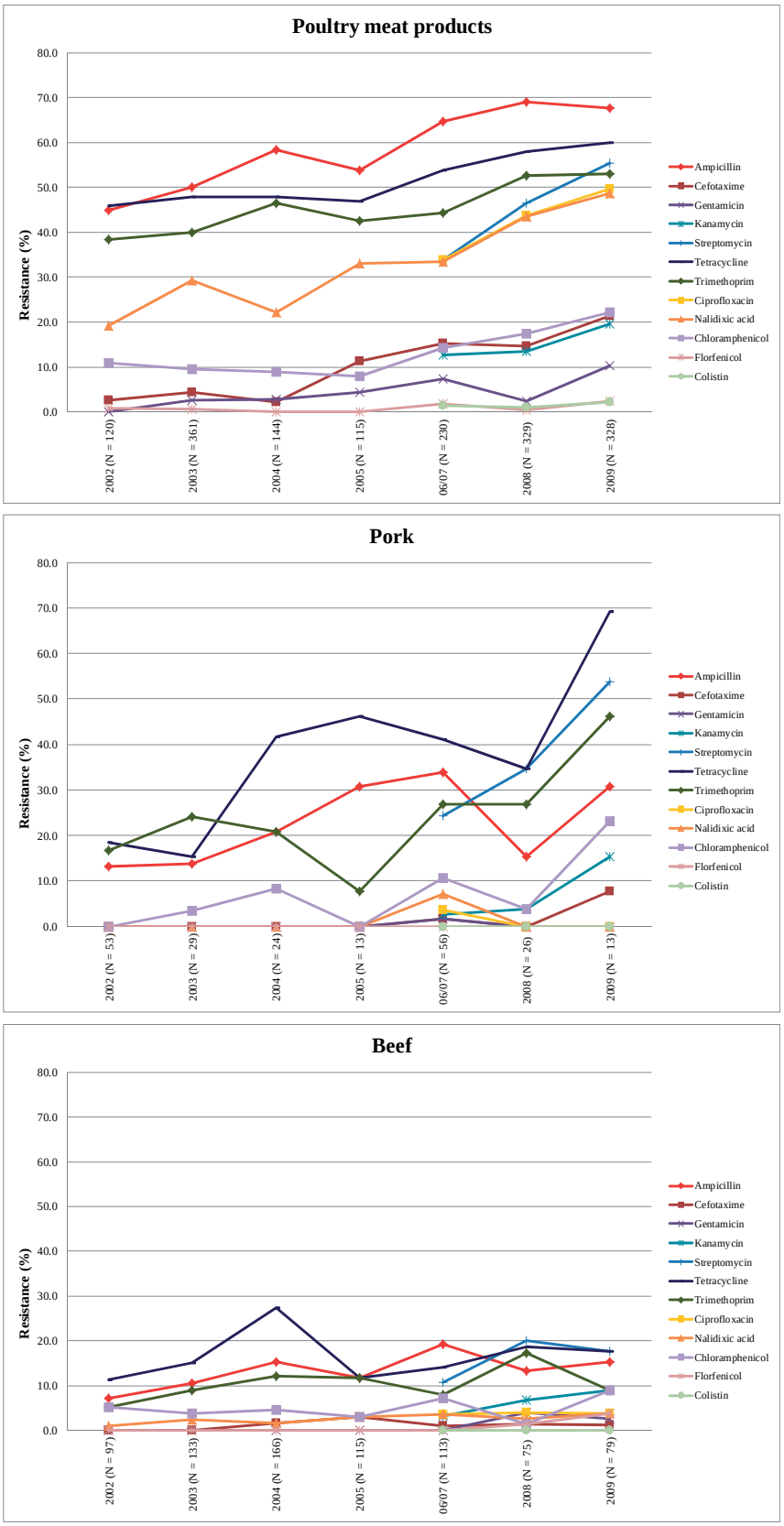
Table 7.2 shows resistance percentages of *E. coli* strains isolated from raw meat products sampled at retail in the Netherlands by the Dutch Food and Consumer Product Safety Authority (VWA).
Overall, resistance percentages of *E. coli* strains isolated from poultry products were similar to those isolated as indicator organisms from faecal samples of Dutch broiler chickens (Tables 7.1 and 7.2). As in previous years, resistance against sulfamethoxazole was higher in isolates from meat products than in isolates from live animals for all three animal species. This may reflect a difference in methodology between the different labs due to the complex nature of reading test results for sulfonamides.
Interpretation of data from both pork and veal is complicated by the low number of isolates from meat products that are tested. This is reflected in the variability in resistance rates over the years as shown in Figure 7.3. 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. Resistance rates of *E. coli* from beef samples are stable over the years.

***E. coli* in other European countries[10]**

Although not from all European countries data are available, in *E. coli* from food producing animals and raw meat, the resistance percentages are among the highest reported in Europe. Of particular concern is the level of resistance to 3rd generation cephalosporins in poultry and poultry meat, for which only Spain reports a higher level of resistance and most other countries report levels between 0 and 3%.

[10] The Community Summary Report. Antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2009.

Figure 7.3. Trends in resistance (in%) of *E. coli* isolated from raw poultry meat products, beef, and pork, in the Netherlands from 2002 – 2009.



Enterococcus faecalis and *Enterococcus faecium*

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. *Enterococcus faecalis* and *Enterococcus faecium* isolates were selected from fecal samples of chickens, pigs, and cattle. Supplementary to isolates from live animals, susceptibility profiles of *E. faecalis* and *E. faecium* isolated from raw meat are presented.
In 2009 MIC values have been determined for 194 *E. faecalis* and 298 *E. faecium* strains isolated from fecal samples of animals at abattoirs as well as for 238 *E. faecalis* and 74 *E. faecium* isolates from different meat samples. In Table 7.3 MIC distributions are summarized for all *E. faecalis* and *E. faecium* strains isolated from live animals. Table 7.4 presents information on resistance rates in different animal species, specified for broiler chickens, slaughter pigs, veal calves and dairy cows. Trends over the years are depicted in Figure 7.4.
Data for 2009 on *E. faecalis* and *E. faecium* from different meats are presented in Table 7.5. as well as in Figure 7.5 (trends in resistance percentages for *E. faecalis* and *E. faecium* from 2003 to 2009).

Highlights

Overall, enterococci displayed high levels of resistance against tetracycline (92.8% for *E. faecalis*, 71.1% for *E. faecium*) and erythromycin (69.6% for *E. faecalis*, 56.7% for *E. faecium*). Also resistance against streptomycin was common in 2009 (51.0% for *E. faecalis*, 41.9% for *E. faecium*). Notable was the increase in resistance against ampicillin among *E. faecium* isolates from pigs, broilers and veal calves.
Resistance was generally higher in *E. faecium* isolates than in *E. faecalis* isolates, while resistance to tetracycline, erythromycin, streptomycin and chloramphenicol was lower.
Vancomycin resistance was observed in low levels in *E. faecium* (1.0%).

Highest resistance levels among the *Enterococcus* species were detected for tetracyclines, against which 92.8% of all *E. faecalis* and 71.1% of *E. faecium* isolates were resistant (Table 7.3.). In *E. faecalis* resistance rates were generally high in all host animal species. However, among *E. faecium* isolates levels varied for different animal species as shown in Table 7.4. Highest level of tetracycline resistance (90.7%) was present in *E. faecium* from slaughter pigs, compared to only 25.0% of *E. faecium* isolated from dairy cows. Levels in *E. faecium* from broiler chickens and veal calves were 76.3% and 60.5% respectively. It is noticeable that in all animal species resistance levels in both enterococcus species show a tendency to increase from 2006/2007 onwards (Figure 7.4). Most striking is the rise in tetracycline resistance in *E. faecalis* isolated from dairy cows (from 23.1% in 2005 to 84.2% in 2009). An exception is tetracycline resistance in *E. faecium* from veal calves, where levels have decreased from 96.2% in 1996/1997 to 60.5% in 2009 (Figure 7.4). It should be noted however, that for some bacterial subspecies/host animal combinations a limited number of data is available, making interpretation more difficult. With respect to *E. faecalis* and *E. faecium* from raw meat, tetracycline resistance is also common, although more variable (Table 7.5 and Figure 7.5). Again this may be due to limited number of isolates. In general, resistance levels seem to be somewhat lower in enterococcal species recovered from raw meat compared those from live animals.

As expected, ampicillin resistance was detected in *E. faecium*. However, in 2009 resistance levels show a marked increase in strains isolated from pigs (45.4%), broiler chickens (40.4%)as well as in veal calves (30.2%) as illustrated in Figure 7.4. Also for *E. faecium* recovered from pork a higher percentage of resistant strains is found in 2009 compared to previous years (Figure 7.5). These findings are in line with the increased resistance seen in *E. coli*.

Resistance against erythromycin in 2009 was commonly seen in both *E. faecalis* (69.6%) and *E. faecium* (56.7%) as summarized in Table 7.3. Over a longer period of time, erythromycin resistance rates are somewhat variable, although different trends are detectable in distinct animal host species (Figure 7.4), probably reflecting usage patterns. Macrolides in poultry, calves and pigs are predominantly used as flock or group treatment versus administration in dairy cows by injection only.
In 2009, resistance against erythromycin is highest in broiler chickens (81.8% for *E. faecalis* and 79.8% for *E. faecium*). Historically, erythromycin resistance rates for *E. faecalis* and *E. faecium* were much higher in veal calves compared to dairy cattle. In 2009, this is still true for *E. faecium*. However a decrease in erythromycin resistance in *E. faecalis* strains from veal cattle combined with an increase in resistance in *E. faecalis* from dairy cows resulted in similar levels in both categories in 2009 (Figure 7.4).

Table 7.3. MIC distributions (in %) for *Enterococcus faecalis* (N=194) and *Enterococcus faecium* (N=298) isolated in food animals in the Netherlands in 2009.

E. faecalis	MIC (%) distribution mg/L																
(n=194)	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	R%	95% CI
Ampicillin			85.6	12.4	1.0	1.0										1.0	0-2.6
Linezolid			21.1	78.4	0.5											0	0-0.02
Tetracyline		4.6	2.6				1.0	28.9	22.7	40.2						92.8	89.2-96.4
Erythromycin			7.2	9.8	13.4	0.5	1.0	3.1	2.1	0.5	62.4					69.6	62.9-75.8
Vancomycin		0.5	41.2	41.2	17.0											0	0-0.02
Ciprofloxacin		9.3	83.0	4.1	2.6			1.0								1.0	0-2.6
Quinu/dalfopristin			0.5	1.5	1.0	39.7	52.6	4.6								0	0-0.02
Salinomycin		10.8	46.9	5.2	31.4	5.7										5.7	2.1-8.2
Streptomycin									3.1	36.1	9.3	0.5	1.5	49.5		51.0	43.8-58.2
Gentamicin					1.0	18.0	74.7	4.1		0.5			1.5			2.1	0.5-4.1
Chloramphenicol					0.5	76.8	3.6	1.0	6.2	11.9						18.0	12.9-23.7
Florfenicol				4.6	95.4											0	0-0.02
E. faecium	**MIC (%) distribution mg/L**																
(n=298)	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**4096**	**R%**	**95% CI**
Ampicillin			22.8	23.5	18.8	28.5			1.3	5.0						34.9	29.4-40.1
Linezolid			2.0	68.1	29.5	0.3										0.3	0-1.0
Tetracyline		28.2	0.7				0.3	2.0	16.8	52.0						71.1	65.9-76.3
Erythromycin			15.8	17.1	10.4	2.3	0.3	0.3	0.3		53.4					56.7	50.8-62.2
Vancomycin		51.3	35.9	8.7	3.0	0.3			0.3	0.3						1.0	0-2.3
Ciprofloxacin		5.7	24.2	21.5	35.2	12.1	1.0			0.3						13.4	9.7-17.4
Quinu/dalfopristin		8.7	10.7	16.8	52.7	9.4	1.0	0.7								80.5	75.6-84.6
Salinomycin		0.3	32.6	25.2	4.7	37.2										37.2	31.8-42.5
Streptomycin							0.3	0.7	38.9	18.1	0.3	0.7	2.3	38.6		41.9	36.1-47.5
Gentamicin					9.7	37.6	39.9	6.7	1.3	2.3			2.3			6.0	3.3-8.7
Chloramphenicol					1.7	66.4	17.8	12.4	1.7							1.7	0.3-3.3
Florfenicol				1.3	91.9	6.4			0.3							0.3	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. The dashed bars indicate clinical breakpoints.

In general, erythromycin resistance in *E. faecalis* and *E. faecium* isolated from fresh meat is lower compared to isolates recovered from fecal samples. However, trends over the years are similar in isolates from both sources.

As in previous years, vancomycine resistance is occasionally present among *E. faecium* strains isolated from animals. In 2009 two highly resistant strains were isolated, from a chicken and from a pig. Both strains were also resistant against tetracycline, erythromycin and quinupristin-dalfopristin. The galline strain showed additional resistance against streptomycin and salinomycine, the porcine strain against ampicillin.

In 2009 again high level ciprofloxacin resistant *E. faecalis* and *E. faecium* isolates were observed (MIC ≥16 mg/l) as shown in Table 7.3. Acquired ciprofloxacin resistance shows some variation among the animal species, and appears to slightly increase over the years as shown in Figure 7.4. Overall, resistance levels are somewhat higher in *E. faecium* compared to *E. faecalis.*

Also resistance against salinomycine is more often encountered in *E. faecium* than in *E. faecalis* (37.2% and 5.7% respectively) as well as for the streptogramin combination of quinupristin and dalfopristin (synercid®) (80.5% versus 0%). This combination is a last resort drug for the treatment of infections caused by staphylococci and vancomycine-resistant *E. faecium* (VRE). Although acquired resistance was observed in a high percentage of *E. faecium* it should be noted that the majority of reduced susceptible strains show MIC values below the clinical breakpoint value of >4 mg/ml. With respect to *E. faecium* strains recovered from meat samples, resistance rates have increased over the years to similar rates as seen in *E. faecium* isolated from live animals.

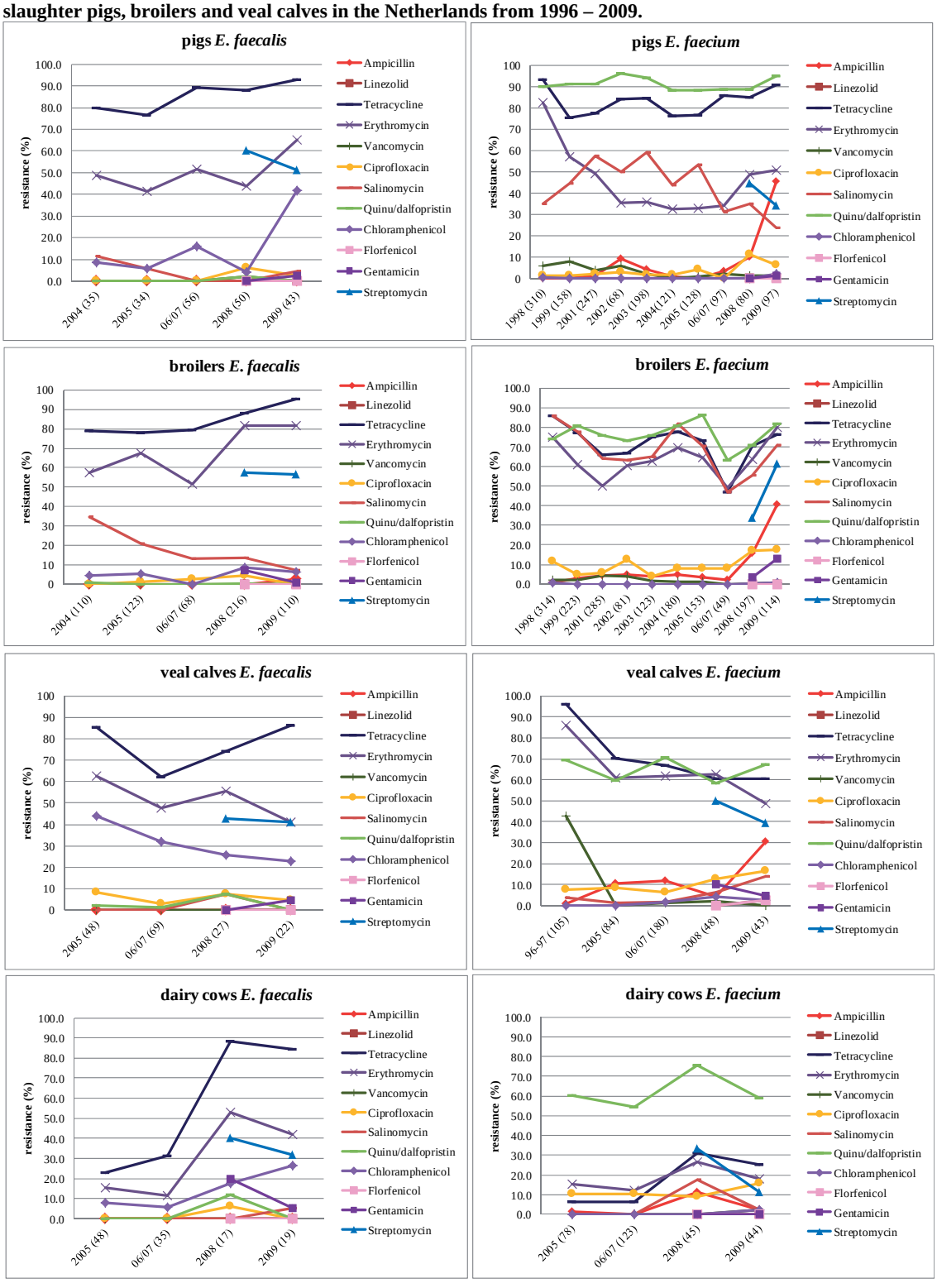
Table 7.4. Resistance percentages (%) of *Enterococcus faecalis* and *Enterococcus faecium* isolated from faeces from slaughter pigs, broilers, veal calves, and dairy cows in the Netherlands in 2009.

E. faecalis	**Slaughter pigs (43)**	**Broiler chickens (110)**	**Veal calves (22)**	**Dairy cows (19)**
Ampicillin	0	1.8	0	0
Linezolid	0	0	0	0
Tetracyline	93.0	95.5	86.4	84.2
Erythromycin	65.1	81.8	40.9	42.1
Vancomycin	0	0	0	0
Ciprofloxacin	2.3	0	4.5	0
Salinomycin	4.7	7.3	0	5.3
Quinu/dalfopristin	0	0	0	0
Gentamicin	2.3	0.9	4.5	5.3
Streptomycin	51.2	56.4	40.9	31.6
Chloramphenicol	41.9	6.4	22.7	26.3
Florfenicol	0	0	0	0
E. faecium	**Slaughter pigs (97)**	**Broiler chickens (114)**	**Veal calves (43)**	**Dairy cows (44)**
Ampicillin	45.4	40.4	30.2	2.3
Linezolid	0	0	2.3	0
Tetracyline	90.7	76.3	60.5	25.0
Erythromycin	50.5	79.8	48.8	18.2
Vancomycin	1.0	0.9	0	2.3
Ciprofloxacin	6.2	17.5	16.3	15.9
Salinomycin	23.7	71.1	14.0	2.3
Quinu/dalfopristin	94.8	81.6	67.4	59.1
Gentamicin	1.0	13.2	4.7	0
Streptomycin	34.0	61.4	39.5	11.4
Chloramphenicol	2.1	0.9	2.3	2.3
Florfenicol	0	0	2.3	0

Resistance against streptomycin is rather common in *E. faecalis* and *E. faecium*, roughly up to 50% of the isolates are resistant, with some variation over the different animal host species. Typically, streptomycin resistant *Enterococcus* strains display high MIC values of >1024 mg/ml.
Gentamicin resistance is occasionally found in both *E. faecalis* and *E. faecium*. Generally, highest levels of resistance against aminoglycoside antibiotics are found in *E. faecium* isolates from broiler (61.4% streptomycin resistant and 13.2% gentamicin resistant). Compared to *E. faecalis* and *E. faecium* from fecal samples, isolates from meat samples usually show lower levels of resistance against aminoglycosides.

Typically *E. faecium* strains are susceptible to chloramphenicol and florfenicol, while among *E. faecalis* chloramphenicol resistance is more commonly detected. In 2009, highest levels were seen in strains isolated from fecal samples from pigs (41.9%), and also regularly detected in bovine samples (22.7% from veal calves and 26.3% from dairy cows). Compared to live animals, levels of chloramphenicol resistance in *Enterococcus* species from meat samples were lower as summarized in Table 7.5.

Figure 7.4. Trends in resistance percentages of *Enterococcus faecium* and *Enterococcus faecalis* isolated from slaughter pigs, broilers and veal calves in the Netherlands from 1996 – 2009.



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

Table 7.5. Resistance % of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from raw meat products from pork, poultry, beef, veal, and lamb in the Netherlands in 2009.

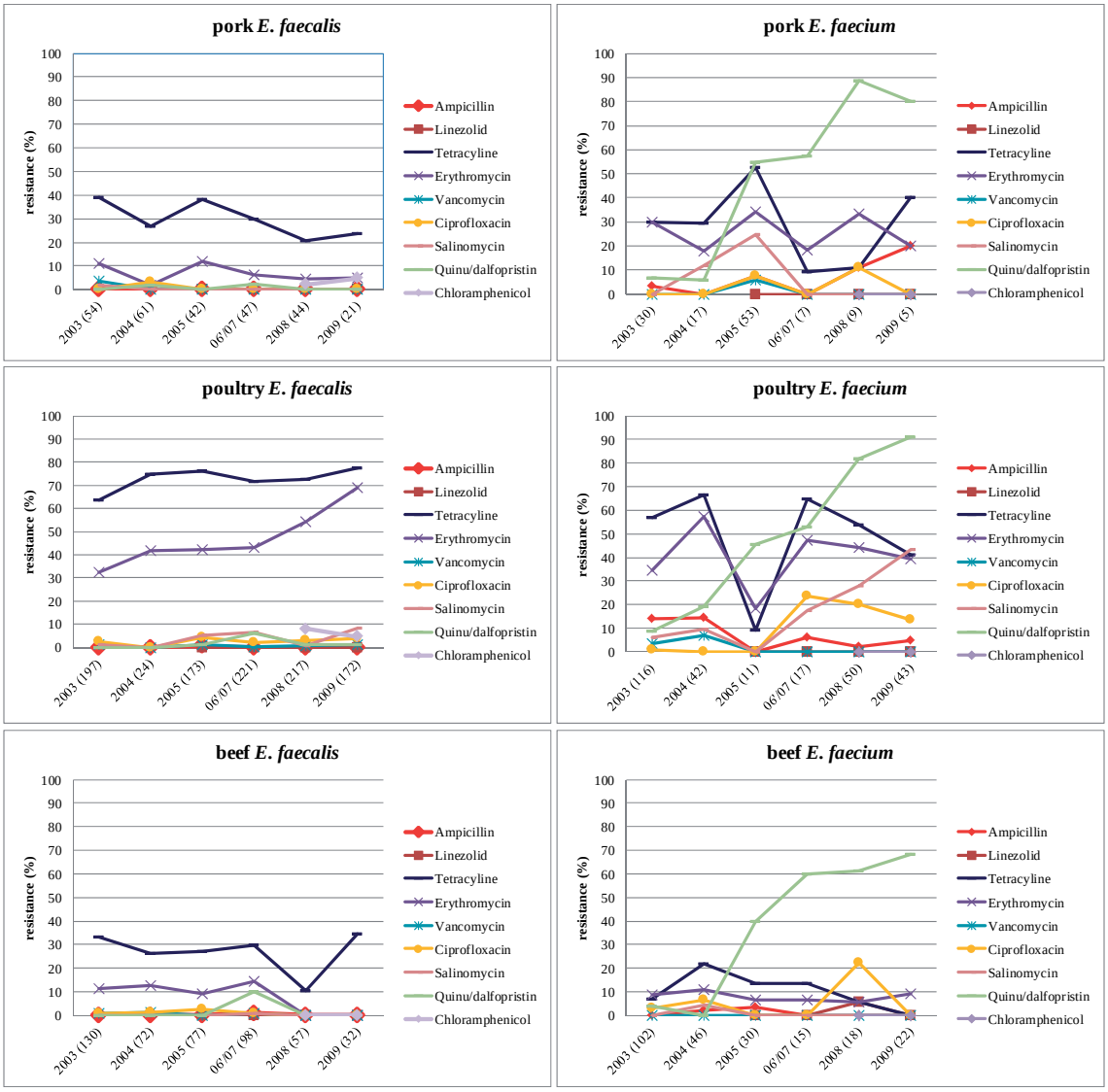
E. faecalis	Pork (21)	Poultry (172)	Beef (32)	Veal (7)	Lamb (6)
Ampicillin	0	0	0	0	0
Linezolid	0	0	0	0	0
Tetracyline	23.8	77.4	34.4	42.9	16.7
Erythromycin	4.8	69.0	0	14.3	0
Vancomycin	0	1.1	0	0	0
Ciprofloxacin	0	4.0	0	0	0
Salinomycin	0	8.5	0	0	0
Quinu/dalfopristin	0	1.1	0	0	0
Gentamicin	0	2.3	0	14.3	0
Streptomycin	4.8	47.5	12.5	28.6	0
Chloramphenicol	4.8	4.5	0	28.6	0
Florfenicol	0	0	0	0	0
E. faecium	**Pork (5)**	**Poultry (43)**	**Beef (22)**	**Veal (3)**	**Lamb (2)**
Ampicillin	20.0	4.7	0	0	0
Linezolid	0	0	0	0	0
Tetracyline	40.0	40.9	0	33.3	0
Erythromycin	20.0	39.5	9.1	33.3	50.0
Vancomycin	0	0	0	0	0
Ciprofloxacin	0	13.6	0	0	0
Salinomycin	0	43.2	0	0	0
Quinu/dalfopristin	80.0	90.9	68.2	100.0	100
Gentamicin	0	3.4	0	0	0
Streptomycin	20.0	31.0	4.5	33.3	0
Chloramphenicol	0	0	0	0	0
Florfenicol	0	0	0	0	0

***Enterococci* in other European countries[11]**

In 2009, information on antimicrobial resistance in enterococci from animals and food from eight European countries showed a wide variation in the levels of resistance. To the same extend as in *E. coli*, the resistance percentages in *Enterococci* from food producing animals in the Netherlands are among the highest reported in Europe.
Especially the high resistance levels observed to macrolides are considered of particular significance because these substances have been defined as critically important antimicrobial in human medicine.

[11]The Community Summary Report. Antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2009.

Figure 7.5. Trends in resistance percentages in *Enterococcus faecalis* and *Enterococcus faecium* isolated from raw meat products from pork, poultry, and beef in the Netherlands from 2003 to 2009.



III Appendices

Appendix I. Prevalence of ESBL and/or AmpC producing *E. coli* in the Dutch broiler production chain

C.M. Dierikx and D.J. Mevius,

Introduction

Since 2003, a dramatic increase in occurrence of cefotaxime-resistant *Escherichia coli* and *Salmonella enterica* isolates from Dutch broiler chickens was observed. In 2008, 15% of *E. coli* randomly isolated from faecal samples using non-selective plates was cefotaxime-resistant, compared to 3% in 2003 (Anonymous, 2010). Genetic analysis of *E. coli* isolates from 2006 demonstrated that the cefotaxime-resistant isolates all harbored a plasmid mediated ESBL ($bla_{CTX-M-1}$, $bla_{CTX-M-2}$, bla_{TEM-52}, bla_{TEM-20}, bla_{SHV-2}) or an AmpC-type beta-lactamase (bla_{CMY-2}) (Dierikx et al., 2010). Information on the prevalence of these ESBL and/or AmpC-producing isolates on Dutch broiler farms was lacking. Therefore in a pilot study on 26 broilers production farms the prevalence of ESBL and/or AmpC producing *E. coli* was determined. From 25 to 41 animals per farm faecal samples were collected from the cloaca using sterile cotton swabs. All farms were found positive and on 85% of the farms in up to 80% of examined broiler chickens ESBL and/or AmpC producing *E. coli* were detected (Dierikx, 2010). To understand more about the spread of ESBL and/or AmpC producing *E. coli* in the Dutch broiler industry, different levels in the broiler production chain were examined for the presence and prevalence of these bacteria.

Methods used

Between July 2009 and January 2011 the Dutch broiler production chain was investigated for the presence of cefotaxime-resistant *E. coli*. Faecal samples were taken from day-old chickens at hatcheries (parents and broiler chickens) or grandparent farms. All samples were inoculated overnight on MacConkey agar with 1 mg/L cefotaxime and in enrichment broth using Luria Bertani (LB) broth + 1 mg/L cefotaxime and incubated aerobically overnight at 37°C. If the following day no growth was observed on the agar plate, 10 µl of the enrichment broth was inoculated on MacConkey agar with 1 mg/L cefotaxime and incubated overnight at 37°C. From each plate one morphologically suspected colony was biochemically confirmed to be *E. coli.* All *E. coli* isolates were tested phenotypically for their susceptibility to cefotaxime, ceftazidime, both as single drugs and as combination disks with clavulanic acid, and cefoxitin according to CLSI M100-S20. Based on the phenotype the isolates were classified as Extended Spectrum Beta-Lactamase (ESBL) or AmpC-producers. As a rule, ESBL-producers are resistant to cefotaxime and/or ceftazidime. One or both of these cephalosporins is synergistic with clavulanic acid and the isolates are susceptible to cefoxitin. AmpC-producers are resistant to cefotaxime and/or ceftazidime, but without synergy with clavulanic acid and are resistant to cefoxitin.

Results

Grandparent day-old chickens

Two breeds of chickens were examined. From breed A, a total of 80 animals were sampled. The animals were derived from eight different production farms (located outside the Netherlands) and from each location 10 animals were screened by sampling caecal content (the animals were sacrificed by the producer for other screening tests). Animals from two production farms were negative. The overall prevalence of ESBL and/or AmpC-producing *E. coli* was 22.5% (Table A1). The prevalence ranged from 0% to 70% per production farm.

For breed B meconium was examined from a total of 125 animals. The animals were derived from five different production farms (located outside the Netherlands), 25 animals per farm. The overall prevalence was 44%, ranging from 36% to 60%.

Parent day-old chickens

Two breeds of parent broiler chickens were examined. From breed A, meconium samples were taken in hatching units from 649 animals derived from nine different production farms. A minimum of 25 samples were taken per production farm. Animals from five farms were found negative for ESBL and/or AmpC-producing *E. coli*. The overall prevalence was 0.9%, ranging from 0% to 4% per production farm.

From breed B meconium of a total of 325 animals, derived from eight different production farms, was examined. From five farms all animals examined were negative for ESBL and/or AmpC-producing *E. coli.* The overall prevalence was 5.8%, ranging from 0% to 13% per production farm.

Day-old broiler chickens

Meconium samples from 425 broiler chickens, derived from 12 different production farms were examined at two hatcheries. Although at both hatcheries animals of breed A and B were produced, only animals from breed A were present at the sampling day. Again a minimum of 25 samples per production farm were examined. Samples derived from animals from seven production farms were negative for ESBL and/or AmpC-producing *E. coli.* The overall prevalence was 3.3%, ranging from 0% to 20%.

Table A1. Results ESBL and/or AmpC-prevalence of day-old animals in the Dutch broiler production chain.

Animals sampled	Breed	Total number of samples	Number of farms of which animals originated	ESBL *E. coli* positive samples	Overall percentage ESBL *E. coli* positive
Day-old grandparents	A	80	8	18	22.5%
	B	125	5	55	44.0%
Day-old parents	A	649	9	6	0.9%
	B	325	8	19	5.8%
Day-old broiler chickens	A	425	12	14	3.3%

Discussion and conclusions

In the Dutch poultry production chain ESBL and/or AmpC producing *E. coli* were present at every level that was examined. Prevalence seems lower in day-old parent chickens and day-old broiler chickens and higher in day-old grandparent chickens which are derived from outside the Netherlands (UK and USA). The low prevalence in day-old broiler chickens is surprising as previous research showed that these animals at 6 weeks of age are almost all positive for ESBL and/or AmpC-producing *E. coli* (Dierikx, 2010).

Grandparents at week 18 and week 31 of age were found positive at moderate level (data not shown) which indicates that especially on Dutch broiler production farms optimum circumstances are present to multiply ESBL and/or AmpC-producing bacteria. Preliminary data on genetic analysis of these isolates show that throughout the production chain $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{CMY} are the predominant beta-lactamase families present.

The data from this study show that ESBL and/or AmpC producing *E. coli* are introduced in the Dutch poultry production chain through imported day-old grandparent chickens. Moreover the data indicate that the occurrence of these organisms in the different levels of the Dutch poultry production chain is the result of vertical transmission, local recirculation and selection. Further research is currently conducted to understand more about the driving forces that lead to a rapid spread of ESBL and/or AmpC producing *E. coli's* in broilers at the broiler production farms.

References

Anonymous. 2010. Monitoring of antibiotic usage and antimicrobial resistance in the netherlands in 2008 (maran-2008).

Dierikx, C., A. van Essen-Zandbergen, K. Veldman, H. Smith, and D. Mevius. 2010. Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. Vet Microbiol 145: 273-278.

Dierikx, C. M., Fabri, T , Goot, JA van der, Molenaar, R, Veldman, KT, Putirulan, FF, Mevius, DJ. 2010. Prevalence of extended-spectrum-beta-lactamase producing *E. coli* isolates on broiler farms in the Netherlands. In: Scientific spring meeting of the Dutch Society for Medical Microbiology and the Dutch Society for Microbiology, Arnhem. p S28 - 29.

Appendix II. Materials and Methods

Salmonella enterica

A total of 21,456 isolates were tested for antimicrobial susceptibility between 1999-2008 (Table A2). Human isolates (N=11,742) 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=1512) and cattle, including calves, (N=749) 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=1558; layers, reproduction animals and eggs, N=899) 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, etcetera.).

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	2009
Human	11742	647	349	1055	862	1338	1339	1176	1273	1089	1502	1112
Pig	1512	31	195	114	168	127	119	120	115	135	335	53
Cattle	749	18	28	56	33	23	106	90	159	87	76	73
Chicken (misc.)	1016	0	10	174	172	160	29	30	116	159	90	76
Broilers (faeces/meat)	1558	68	110	143	212	206	110	82	164	54	303	106
Layers/Repro/Eggs	899	93	86	62	56	88	91	232	75	44	37	35
Other sources	3980	0	9	309	330	446	473	603	535	451	378	446
Total	21456	857	787	1913	1833	2388	2267	2333	2437	2019	2721	1901

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.

***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 by the Food and Consumer Product Safety Authority as part of the national control programs. Samples were taken at slaughterhouses (pigs and poultry samples), or at farms (veal calves and dairy cattle). For isolation of the above mentioned organisms one faecal sample was taken for each epidemiological unit (farm, flock or group of animals), or the caeca collected (broilers). At the laboratory the samples were 1:10 (w/v) diluted directly in buffered peptone solution with 20% glycerol and stored at –20°C. After arrival of the samples, isolation of *E. coli*, *E. faecium*, *E. faecalis* and *Campylobacter* spp. was performed without delay 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[12].
For isolation of *Campylobacter* CCDA-agar with 32 µg/ml cefoperazone and 10 µg/ml amphotericin B to

[12] Dutka-Malen, S., S. Evers, and P. Courvalin, *Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR.* J Clin Microbiol, 1995. **33**(1): p. 24-7.

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.

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 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.

Shiga 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.

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[13][14] (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 epidemiological 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.

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

[14] 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	-	-
Quinupristin-dalfopristin	-	-	-	-	1	32
Salinomycin	-	-	-	-	4	4
Streptomycin	32[a]	16	2	4	128	512
Sulfamethoxazole	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

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