MARAN 2003

Monitoring of Antimicrobial Resistance
and Antibiotic Usage in Animals in the Netherlands
In 2003

Contents

Summary

MARAN 2003 presents data on resistance in zoonotic and commensal bacteria of animal origin and animal pathogens, and data on usage of antimicrobials in food producing animals.

In 2003 the total amount of therapeutic use of veterinary antibiotics has decreased by 2% since 2002. However, in 2003 the numbers of kg's of food animals produced in The Netherlands have decreased as well. The main antibiotic classes used in food producing animals were as before tetracyclines and trimethoprim-sulphonamide combinations. Quinolone usage was 5000 kg in 2003, 272 kg of which were fluoroquinolones used in poultry. The use of antimicrobial growth promoters decreased from 250.000 kg in 1998 to 75.000 kg in 2003.
Comparing usage data from The Netherlands with data from Denmark shows that the therapeutic use of antibiotics in pig, cattle and broiler production is more intensive in The Netherlands. The reason why in Denmark fewer antibiotics are used for therapeutic purposes is subject for debate. Data from this report indicate that in The Netherlands the usage of antibiotics in veal calves, an industry that is lacking in Denmark and the fact that in Denmark in pigs more highly potent macrolides administered in lower dosages are used than in The Netherlands are major factors in explaining the observed difference. Other factors that may be involved are the different distribution system of antibiotics in Denmark, the lower incidence of diseases and the fact that virtually every administration of antibiotics is monitored in Denmark which may have a positive effect on the prescription frequency of \Danish veterinarians.

Data from 2003 in the current report indicate a slow overall increase in quinolone (nalidixic acid/flumequin) resistance (all nalidixic acid/flumequin-resistant strains demonstrate decreased susceptibility to ciprofloxacin) in salmonella's in The Netherlands. In *Salmonella* Typhimurium quinolone resistance was limited to isolates from human infections and poultry products. In *S.* Java, the very rapid increase in quinolone resistance from 0% in 2000 to 35% in 2002 stabilised in 2003 (34,2%) in strains from broilers. None of these isolates demonstrated high level ciprofloxacin resistance as was observed in Germany.
In *Salmonella* Enteritidis a major increase in quinolone-resistance was observed, predominantly in human isolates of Pt1, 6a, 8, and 14b related to imported products and travel. In Dutch layers no quinolone resistant *S.* Enteritidis strains were detected.
Ciprofloxacin-resistance in *Campylobacter jejuni* strains from broilers in 2003 slightly increased to 41,7%. The ciprofloxacin resistance percentages in human clinical isolates were stable at 31%. For acquisition of human infections with fluoroquinolone-resistant campylobacters, travel contributed to a higher proportion than domestic acquisition.
Quinolone resistance in food borne commensal organisms was mainly observed in *Escherihia coli* from broilers and broiler products. In this animal species approximately 35% of all *E. coli*'s demonstrated reduced susceptibility to ciprofloxacin and 3% high level resistance to this drug. In pigs no quinolone resistance was observed in *E. coli.* As in *Salmonella* Java and *C. jejuni*, this reflects the selection pressure through usage of enrofloxacin and flumequin in broilers.
Resistance to cefotaxime (ESBL-positive) was found, both in human and poultry salmonella's (N = 13). The majority of these isolates were multiple resistant to amoxicillin, cefotaxime, tetracycline, trimethoprim, sulphamethoxazole and chloramphenicol. Six of these isolates were also resistant to nalidixic acid.
In randomly picked *E. coli* strains from broilers cefotaxime-resistance was present less frequently than in 2002 (2,4% versus 6,1% in 2002). In broilers four cefotaxime resistant strains were isolated that were suspected to be ESBL-positive, and in pigs one.

Resistance to vancomycin in *Enterococcus faecium* was low and showed a tendency to further decrease. Resistance to the other growth promoters stabilized after the initial decrease due to the ban of these products in 1999. Resistance to avilamycin increased again in 2003.
In the foodborne commensal organisms of the animal species included, the percentages of multiple resistant strains were much higher in broilers than in pigs. This reflects both the intensive use of antibiotics in broilers and the fact that measurement of antimicrobial resistance followed much closer after treatment in broilers (at the most 6 weeks) than in other food animals. Multiple resistance was more present in *Pasteurella multocida* and *Mannheimia haemolytica* from veal calves than from dairy cattle, again reflecting the different use practices in these animals.

Comparing the resistance data from this report with DANMAP 2003 demonstrates that in general in Dutch strains of foodborne pathogens and commensals the resistance levels are higher than in Danish strains. It may reflect the higher consumption pattern in The Netherlands.

As in DANMAP 2003, travel associated resistance also plays an important role in our data. In addition, the import of resistant strains on meat products is documented in DANMAP 2003. It stresses the necessity for The Netherlands to focus further on imported products in an attempt to quantify the contribution of the imported products to the resistance situation in The Netherlands.

Conclusions and recommendations
It can be concluded that the level of usage of antibiotics in food animals in The Netherlands is higher than in a country with a similar but less intensive husbandry system. Moreover, in Dutch bacteria from food animals the resistance levels are higher.
Data from the present report indicate that the major risk factors for development of antimicrobial resistance of public health concern are antibiotic usage in poultry (both in The Netherlands and abroad), consumption of imported products and travel.

Based on the results of this report it can be recommended that:
- Usage and resistance data from veal calves need to be included in future reports;
- Resistance trends of quinolones in Salmonella, Campylobacter and *E. coli* in food animals and in humans warrant optimisation of prudent use policies;
- Imported food products of animal origin should be included in the monitoring programme;
- More detailed data on therapeutic use of antibiotics in the different food animal species (pigs, poultry and veal calves) are needed to determine the real exposure to antibiotics.

Samenvatting

MARAN 2003 bevat gegevens over resistentie in zoönotische en commensale bacteriën van dierlijke oorsprong en gegevens over het gebruik van antimicrobiële stoffen in voedselproducerende dieren.

In 2003 is de hoeveelheid therapeutisch gebruikte antibiotica in vergelijking met 2002 met 2% afgenomen. Echter, in 2003 zijn in Nederland het aantal kilo's geproduceerde landbouwhuisdieren ook afgenomen. De meest gebruikte klassen antibiotica in landbouwhuisdieren waren als tevoren tetracyclinen en trimethoprim/sulfonamiden combinaties. Het gebruik van quinolonen bedroeg 5000 kg in 2003, waarvan 272 als fluoroquinolonen in pluimvee (vnl. vleeskuikens). Het gebruik van antimicrobiële groeibevorderaars nam af van 250.000 kg in 1998 naar 75.000 kg in 2003.
In de landbouwhuisdierenproductie worden in Nederland meer antibiotica als therapeuticum gebruikt dan in Denemarken. Data in dit rapport maken het aannemelijk dat het gebruik van antibiotica in vleeskalveren, een sector die in Denemarken niet bestaat en het veelvuldige gebruik van potente macroliden in varkens in Denemarken een bijdrage leveren aan het verschil. De macroliden worden lager gedoseerd dan de veelgebruikte tetracyclinen in Nederland. Ook het verschil in distributiesysteem van antibiotica, de lagere incidentie van dierziekten en het feit dat werkelijk al het gebruik in Denemarken wordt gemonitord, wat een rem kan zijn op het voorschrijfgedrag van dierenartsen, kunnen verklaringen zijn voor het verschil.
De data uit 2003 in dit rapport geven aan dat er een langzame toename in resistentie tegen quinolonen (flumequine/nalidixinezuur) waarneembaar is in salmonella 's geïsoleerd in Nederland (deze stammen zijn ook allemaal minder gevoelig voor ciprofloxacin). In *Salmonella* Typhimurium werd quinolonen resistentie alleen waargenomen in humane klinische isolaten en isolaten uit vleesproducten van pluimvee. In *Salmonella* Java uit slachtkuikens stabiliseerde de snelle toename in flumequine resistentie van 2000 tot 2002 (0% - 35%) in 2003 (34,2%). Er werden geen ciprofloxacin resistente *S.* Java's waargenomen in tegenstelling tot Duitsland. In *Salmonella* Enteritidis werd de grootste toename in quinolonen resistentie waargenomen, voornamelijk in humane isolaten behorend tot de faagtypen pt1, 6a, 8 en 14b, gerelateerd aan consumptie van geïmporteerde dierlijke producten en buitenlandse reizen. In Nederlandse leghennen werden tot op heden geen quinolonen resistente *S.* Enteritidis isolaten gevonden.
Ciprofloxacin-resistentie in *Campylobacter jejuni* stammen uit vleeskuikens nam in 2003 iets toe tot 41,7%. De ciprofloxacin resistentie percentages in humane klinische isolaten waren stabiel op 31%. Voor het verkrijgen van humane infecties met fluoroquinolonen-resistente campylobacters, was het reizen naar het buitenland een belangrijker risicofactor dan in Nederland opgedane infecties.
Quinolonen resistentie in commensale darmflora werd voornamelijk waargenomen in *E. coli* uit vleeskuikens en filets. In deze diersoort waren ongeveer 35% van alle *E. coli*'s verminderd gevoelig voor ciprofloxacin en 3% resistent. In vleesvarkens werden geen quinolonen resistente *E. coli'*s gevonden. Net als in *S.* Java en *C.* jejuni is dit een afspiegeling van de selectiedruk door het gebruik van flumequine en fluoroquinolonen in vleeskuikens in Nederland.
Resistentie tegen cefotaxime (ESBL-positief) werd zowel in humane salmonella's als in salmonella's uit pluimvee waargenomen (N = 13). De meerderheid van deze isolaten was multiresistent tegen amoxicilline, cefotaxime, tetracycline, trimethoprim, sulfamethoxazole en chlooramfenicol. Zes van deze isolaten waren ook resistent tegen quinolonen (nalidixinezuur).

In commensale *E. coli*'s uit vleeskuikens kwam cefotaxime resistentie minder vaak voor dan in 2002. (2,4% versus 6,1% in 2002).
Resistentie tegen vancomycine in *Enterococcus faecium* kwam weinig voor en vertoont een neergaande tendens. Resistentie tegen de andere groeibevorderaars stabiliseerde na de initiële afname na het verbod in 1999. Resistentie tegen avilamycine nam weer wat toe in 2003.

In de commensale darmflora van de onderzochte diersoorten waren de percentages multiresistente stammen veel hoger in vleeskuikens dan in vleesvarkens. Dit is zowel een afspiegeling van het gebruik in die dieren, maar ook van de houderijsystemen. In vleeskuikens volgt het moment waarop de resistentie wordt gemeten veel korter op het tijdstip van antibacteriële therapie (maximaal 6 weken) dan in varkens. In *Pasteurella multocida* en *Mannheimia haemolytica* van vleeskalveren kwam meer multiresistentie voor dan deze species uit overige kalveren, weer een afspiegeling van het verschil in selectiedruk door antibioticagebruik.
Een vergelijking van resistentiegegevens uit dit rapport en Deense gegevens in DANMAP 2003 laten zien dat in Nederlandse voedselpathogenen en commensalen meer resistentie voorkomt, mogelijk als gevolg van de grotere selectiedruk in Nederland.
Net zoals in DANMAP 2003 wordt vermeld speelt ook in Nederland de reisgerelateerde resistentie een belangrijke rol. In DANMAP 2003 zijn ook gegevens weergegeven over resistente stammen geïsoleerd van importproducten. De cijfers maken de noodzaak duidelijk om ook in Nederland importproducten te gaan screenen op resistente stammen in een poging om de bijdrage van importen aan de resistentiesituatie te kunnen kwantificeren.

Conclusies en aanbevelingen
Therapeutische antibiotica worden in Nederland intensiever gebruikt dan in Denemarken, een land met een vergelijkbare maar minder intensieve dierhouderij. In Nederlandse bacteriën van landbouwhuisdieren komt meer antibioticumresistentie voor.
De gegevens uit dit rapport laten zien dat de belangrijkste risicofactoren voor de ontwikkeling van antibioticumresistentie welke mogelijk van belang zijn voor de volksgezondheid zijn het gebruik van antibiotica in pluimvee (ook in het buitenland), consumptie van geïmporteerde dierlijke producten en buitenlandse reizen.

Gebaseerd op de resultaten van dit rapport kunnen de volgende conclusies worden getrokken:
- Gebruiks- en resistentiegegevens van vleeskalveren moeten worden toegevoegd in toekomstige rapportages.
- De waargenomen toenemende resistentie tegen quinolonen in Salmonella, Campylobacter en *E. coli* geïsoleerd uit voedselproducerende dieren maken het noodzakelijk dat het antibioticumbeleid wordt aangescherpt
- Geïmporteerde dierlijke producten dienen in de toekomst een onderdeel te vormen van het monitoringsprogramma;
- Gedetailleerde gegevens over het therapeutische gebruik van antibiotica in voedselproducerende dieren (varkens, pluimvee en vleeskalveren) zijn nodig om de ware blootstelling aan antibiotica te kunnen vaststellen.

I Usage of antibiotics in animal husbandry in the Netherlands

Usage of Antibiotic Growth Promoters (AGPs) and coccidiostats

Manufacturing, distributing and selling of animal feed containing antibiotic growth promoters (AGPs) and coccidiostats is in the hands of the feed industry and is not controlled by veterinarians. In 1998 250.000 kg of antibiotics were used as AGPs in the Netherlands. Since cross resistance occurs between antibiotics used as AGP and antibiotics used therapeutically for animals and humans, the use of antibiotics as AGP is put under pressure. Since 1999 only few antibiotics are still allowed and used as AGP. These are flavophospholipol (a glycolipid), avilamycin (an orthosomycin), salinomycin and monensin (ionophores). The latter are used both as AGP and as coccidiostat. Based on data provided by manufacturers of feed additives it is calculated that the use of AGPs in 2003 decreased to 75.000 kg. This is a reduction of 70% compared to 1998.

Usage of antibiotics as medicines for therapeutic purposes

Total sales, provided by the pharmaceutical industry

Since 1990 the therapeutic use of antibiotics in the Netherlands has been monitored, based on total sales generously provided by FIDIN (manufacturers and importers of veterinary medicines in the Netherlands). In table 1 most recent data (2003) are shown. Sales from 1997 to 2003, expressed in kg, and the relative contribution of each therapeutic group are summarized in figure 1. Livestock statistics are shown in figure 2.
Comparing 2003 with 2002, total sales of antibiotics decreased by 2%. Because pigs, broilers and veal calves are the animal species to which most antibiotics for therapeutic use are administered, it is relevant to relate the sales figures with the population statistics for these animals. Comparing 2003 with 2002, the pig population decreased by 4%, and the veal calf population increased by 3%. The population statistics for broilers were influenced by an outbreak of avian influenza in the spring of 2003: more than 20 million birds were culled, followed by a period with empty housings. According to the agricultural census in May the broiler population was reduced by 23 %. Information from KIP (poultry production monitoring system, comparing January 1st 2004 with January 1st 2003) confirms a population reduction, but only 8,3% for broilers. Not only the number of birds present was reduced, also the life weight production of poultry was significantly lower (25%, source: Dutch Product Boards for Livestock Meat and Eggs (PVE)).
The outbreak of avian influenza apparently did not cause a substantial change in antibiotic sales as the birds that were culled may have received antibiotics but were not counted as life weight produced. As a result of transportation restrictions of live poultry, housings were overpopulated, possibly resulting in higher individual consumption of antibiotics at these farms.
In the period from 1997 to 2003 total sales of antibiotics for therapeutic use have increased from 332.000 kg to 394.000 kg in 2003 (19 %). The veal calf population in this period increased (4%) and so did the broiler population (21%, based on poultry population in 2002). In this time period the pig population decreased by 21%.
In general the relative contribution of different antibiotic-classes to total sales has remained stable over the years. In 2003 tetracyclines and trimethoprim/sulphonamide combinations

represented 81% (317.000 kg) of the weight of total sales in antibiotics, in 1997 both classes represented 75%.
Quinolone sales (5.000 kg) represented 1% of total sales and this figure has a tendency to decrease. As quinolones have a high potency compared to other antibiotics, the relative use (number of dosages) will be more than 1%. Quinolone sales reported include the use of flumequine as well as more recently developed fluoroquinolones (enrofloxacin, danofloxacin, marbofloxacin, difloxacin). In 2003, 272 kg of these newer fluoroquinolones were used in poultry (FIDIN, unpublished data).
Macrolides sales (18.000 kg) represented 4% of total sales. After the partial ban of AGPs in 1999, sales of macrolides for therapeutic use increased by 58% in 2000 as compared to 1999. In the years 2001 and 2002 sales also increased. In 2003 however, sales of macrolides decreased by 11%.

As the price-level in the Netherlands is low, emigrated farmers from the Netherlands to Denmark and Canada import antibiotics licensed in the Netherlands. The extent is not known but is not considered to be a major factor affecting antibiotic sales data in the Netherlands.

In appendix II a comparison between antibiotic usage in Denmark and the Netherlands is made.

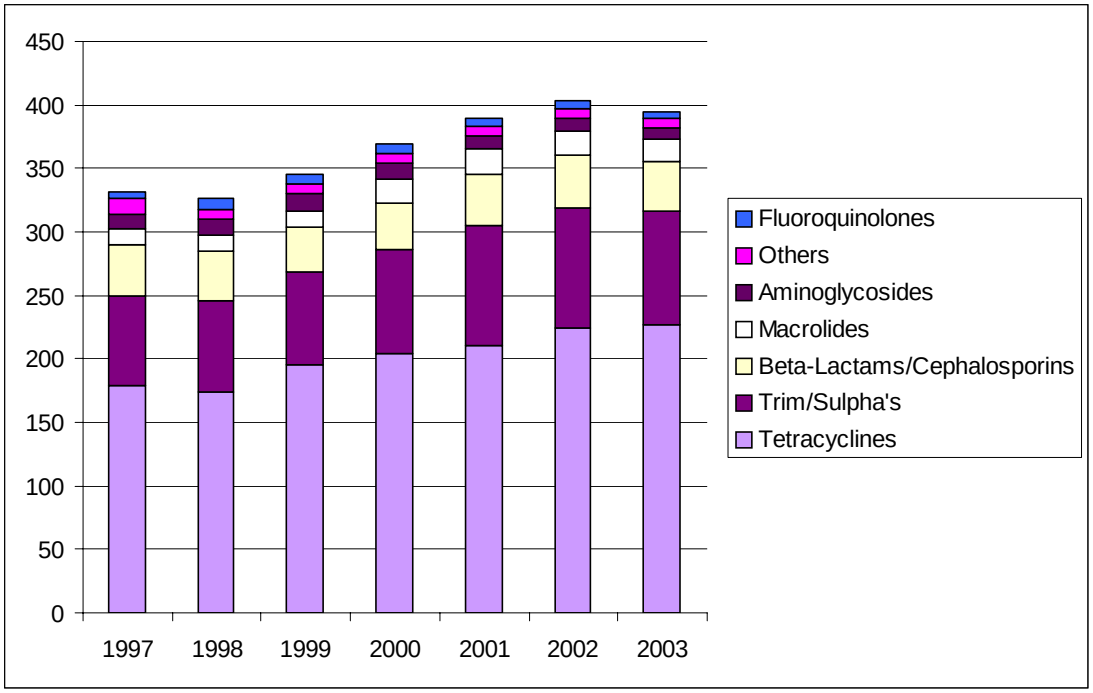
Acknowledgement
The manufacturers and importers of veterinary medicines in the Netherlands, organized in the FIDIN, and the Dutch product board for livestock, meat and eggs (PVE) have been very helpful in providing information.

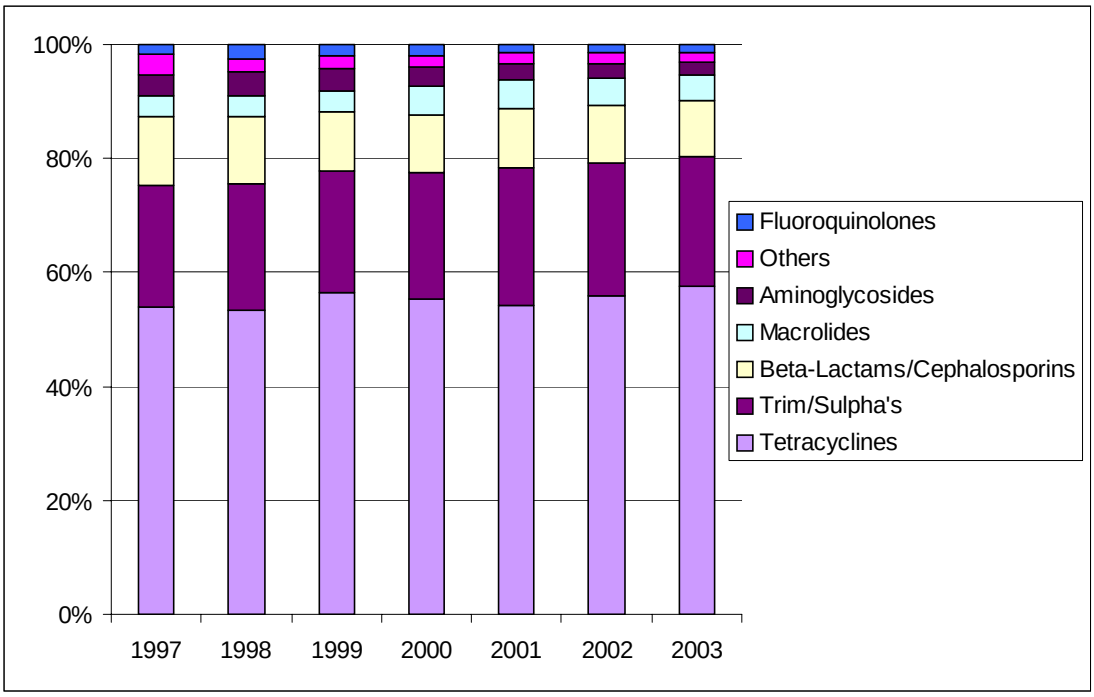
Table 1. Total sales of antibiotics in 2003 in the Netherlands.

	Kg of active substance in 2003	% Difference with 2002	% Of total use
(Fluoro)quinolones	5.000	- 2 %	1 %
Other	7.000	- 9 %	2 %
Aminoglycosides	9.000	- 12 %	2 %
Macrolides	18.000	- 11 %	4 %
Penicillines/cephalosporines	38.000	- 6 %	10 %
Trimethoprim/sulphonamides	90.000	- 4 %	23 %
Tetracyclines	227.000	1 %	58 %
Total	394.000	- 2 %	100 %

Source: FIDIN.

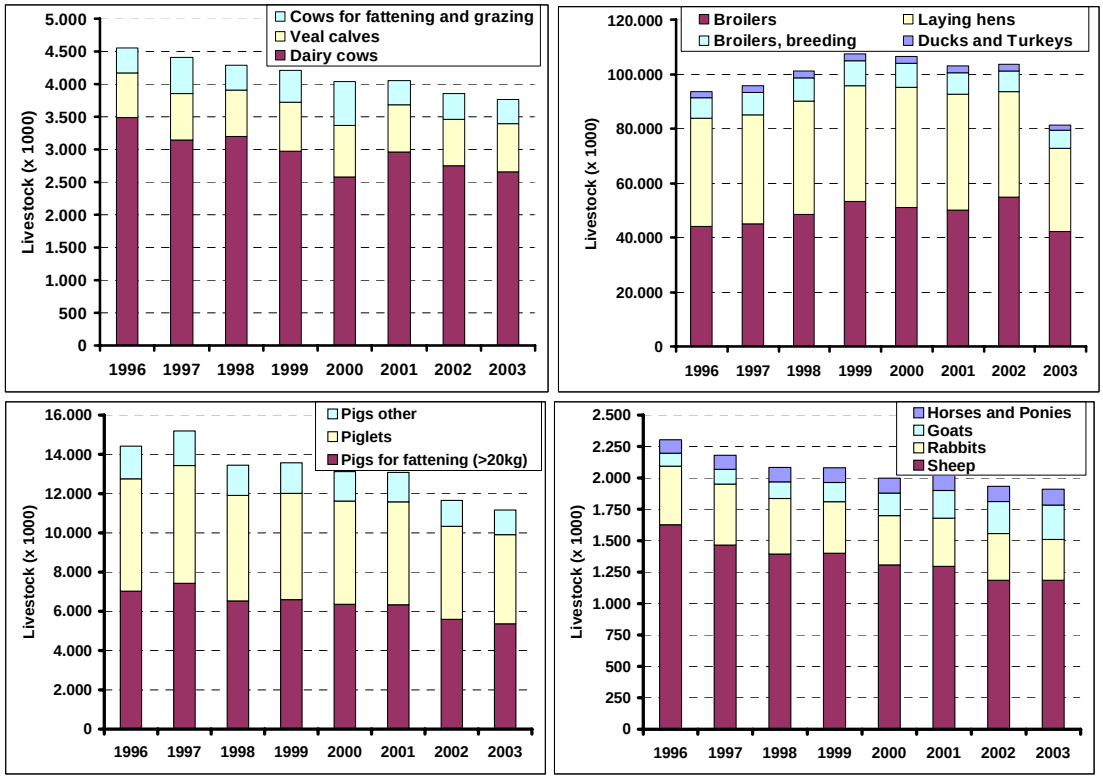
Figure 1 Absolute and relative use of antibiotics for therapeutic use (active ingredient x 1000 kg) in The Netherlands.





Source: FIDIN

Figure 2. Livestock (x 1000) in the Netherlands from 1997 - 2003.



Source: Agricultural census, Central Bureau of statistics (CBS).

II Resistance data

In this chapter susceptibility test results are presented as determined in 2003 for the food-borne pathogens *Salmonella, Campylobacter* and *Escherichia coli* O157, the food-borne commensal organisms *E. coli*, *Enterococcus faecium* and *E. faecalis*, the bovine respiratory disease pathogens *Pasteurella multocida* and *Mannheimia haemolytica* and the bovine mastitis pathogens *Staphylococcus aureus, Streptococcus uberis/parauberis, S. dysgalactiae, E. coli* and coliform bacteria..

Food-borne pathogens

Salmonella spp.

In this chapter resistance percentages are presented on salmonella's isolated from humans with clinical infections: food-animals and their products as potential sources for distribution to humans via the food chain and animal feeds as potential source for food-animals and their food products. For the purpose of antimicrobial resistance surveillance in *Salmonella* spp., it is essential to include information on the relative importance of the different serotypes in humans and food-animals (table 1). In 2003, like in former years, *S.* Typhimurium and *S.* Enteritidis were by far the most frequently isolated serotypes of *Salmonella* in humans in The Netherlands. In pigs *S.* Typhimurium and in cattle *S.* Typhimurium and *S.* Dublin were the most prevalent serotypes. In poultry a difference existed in prevalence of *Salmonella* spp. between broilers and layers. In broilers *S.* Paratyphi B var. Java (*S.* Java) and *S.* Infantis, and in layers *S.* Infantis and *S.* Enteritidis were the predominantly isolated serotypes.
Travel contributed to 8% of the cases of salmonellosis. Among the most frequently isolated human serotypes travel contributed substantially more to the incidence of *S.* Enteritidis than for *S.* Typhimurium.
In 2003 *S.* Typhimurium DT104 was less frequently isolated from humans and cattle but more frequently from pigs. Although the incidence of *S.* Enteritidis clearly increased in humans, this serotype was less frequently isolated from Dutch layers and eggs, indicating another source for the human infections in 2003. The occurrence of *S.* Java in poultry remained stable. The increase in prevalence of *S.* Infantis and *S.* Senftenberg in layers in 2003 is striking, however, this has not resulted in an increase in human infection with these serotypes.

Table 1. Most prevalent *Salmonella* sero-, and phagetypes isolated in 2003 (2002 between brackets) from humans, pigs, poultry, broilers and layers[1] and the % travel related infections in 2001 – 2003.

		Humans	Pigs	Cattle	Poultry	Broilers	Layers
Total number sent to RIVM		2142	278	120	1659	603	239
Serotype/Phagetype	% Travel	% of total number sent to RIVM in 2003 (2002)					
Typhimurium	2%	24	55,4(45,4)	21,7(32,6)	2,2	3,5	1,3
DT104	2%	7,4(10,3)	19,8(13)	8,4(22,9)	1,1	2,5	0(2,2)
Ft507	1%	6,1(4)	9(6,1)	3,3(0,6)	0,2	0,2	0,0
Enteritidis	7%	55,2(44,5)	0,4	3,3	7,7	5,3	25,9(41,8)
Pt 4	6%	19(22,7)	--	--	3,0	1,7	7,5(21,7)
Pt 21	4%	12,2(7,5)	--	--	1,1	1,0	0,5(2,4)
Pt 1	14%	7,9(4,1)	--	--	0,5	1,0	0,5(5,3)
Pt 6	7%	2,8	--	--	1,2	0,3	3,5
Pt 8	4%	4,6(1,8)	--	--	0,5	0,3	0,7
Pt 14b	16%	2,4(1)	--	--	0,2	0	0,7
Pt 7	10%	0,4	--	--	0,3	0,2	1(5,3)
Pt 6a	12%	1,4(0,4)	--	--	0,1	0,3	0
Paratyphi B v Java	5%	0,1	0,4	--	40,1	56,4	3,3
Dublin	4%	0,2	--	59,2	--	--	--
Infantis	9%	1,3	1,4	--	29,7(12,6)	17,9	33,9(16,8)
Senftenberg	19%	0,1	0,7	--	3,4	1	13,8(2,2)
Livingstone	5%	0,5	7,6	2,5	0,5	--	2,5
Derby	5%	0,8	7,9	0,8	0,5	1	--
Goldcoast	1%	2,1	6,1	--	0,1	0,2	--
Brandenburg	4%	0,9	4,3	1,7	0,1	0,3	--
Agona	17%	0,2	0,7	--	1,5	0,7	4,2
Virchow	25%	1	0,4	--	2,4	3,5	0,8
Mbandaka	31%	0,1	--	0,8	2,2	1,7	2,5
Montevideo	26%	0,2	--	3,3	0,4	0,2	--
Heidelberg	4%	0,5	0,4	--	0,8	0,7	1,3
Hadar	26%	1	0,7	--	0,4	0,5	0,4
Anatum	29%	0,2	1,1	--	0,6	0,5	0,8
Indiana	0%	0,1	--	--	1	2	0,4
SI 4,5,12:d:2ef nat	--	--	2,5	--	0,1	--	--
Havana	13%	0,1	0,4	1,7	0,4	0,3	--
Saintpaul	18%	0,1	--	--	0,5	--	2,1
Panama	7%	0,3	1,1	--	0,3	0,3	0,4
London	7%	0,3	1,8	--	--	--	--
SI 9,12:NM	0%	0,2	0	1,7	--	--	--
Typhi	35%	1,8	--	--	--	--	--
Gallinarum	--	--	--	--	0,3	--	1,3
Manhattan	7%	0,1	0,4	0	1,9	0,5	--
Other serotypes		8,6	6,3	3,3	2,9	3,5	5,1

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

[1] Report on trends and sources of zoonotic agents in the EU, 2003, The Netherlands

Table 2. MIC distribution (in %) for all salmonella's (N = 2428) tested for antibiotic susceptibility in 2003.

	MIC distribution (µg/ml)																	
2003	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**R%**	
Amoxicillin						13,3	66,6	3,0	0,1				13,7	3,2				16,9	
Cefotaxim				89,4	8,8	1,2	0,04		0,1		0,2	0,2						0,5	
Imipenem				58,0	39,4	2,4	0,2											0	
Gentamicin					48,9	41,8	7,0	1,2	0,04		0,7	0,2	0,2					1,1	
Neomycin							89,7	8,7	0,7				0,2	0,3	0,2			0,7	
Doxycycline					0,1			21,2	54,8	9,0	5,6	6,6	2,7					23,9	20,9
Tetracycline						0,2	13,7	58,4	10,4	0,7	0,1	3,0	3,6	9,9				16,6	
Trim/Sulpha					84,2	1,9	0,2	1,2	0,8	0,1			11,8					12,6	
Sulphamethox.										25,4	47,9	4,8	1,2	0,2		20,5		20,5	
Trimethoprim						78,1	7,6	1,0	0,3					12,9				12,9	
Ciprofloxacin			88,3	2,6	5,6	2,3	1,0	0,1										0	
Flumequin						12,7	67,4	6,9	0,8	1,3	8,1	1,7	1,0					12,1	11,2
Nalidixic Acid								5,0	78,1	8,9	0,8	0,4			6,8			7,2	
Chloramphenicol									8,7	78,4	6,1	0,5	0,2	1,6	4,6			6,8	
Florfenicol								0,9	66,7	25,1	2,7	4,0	0,2	0,3	0,04			4,6	

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

Table 2 presents MIC-distributions and resistance percentages of all salmonella's tested for susceptibility in 2003. During 2003 the panel of antibiotics in the microtitre trays was changed. Doxycycline was replaced by tetracycline because with the latter antibiotic resistant and susceptible subpopulations of Salmonella (and *E. coli*) can be identified more easily. Flumequin was replaced by nalidixic acid because it improves the comparability with data from similar reports like DANMAP, SVARM and NORMVET. Sulphamethoxazole replaced its combination with trimethoprim because testing the individual drug of this combination provided more reliable information about resistances to these drugs. For the tetracyclines and the quinolones both the resistance percentages for the individual drugs and the overall resistance percentages are presented in the table.
Highest levels of resistance were observed for amoxicillin, doxycycline/tetracycline, sulphamethoxazole, trimethoprim and flumequin/nalidixic acid. Thirteen cefotaxime resistant strains were found. These isolates belonged to the following serotypes: 4 *S.* Virchow, 2 *S.* Paratyphi B var. Java and 1 *S.* Bareilly from poultry, 2 *S.* Typhimurium ft 507 and 508, respectively (Dutch phage typing system), 2 *S.* Concord and 1 *S.* Enteritidis pt4 isolated from human patients and 1 *S.* Enteritidis pt21 from an unknown source. The majority of these isolates were multiple resistant to amoxicillin, cefotaxime (ESBL-positive), tetracycline, trimethoprim, sulphamethoxazole and chloramphenicol. Six of these isolates were also resistant to nalidixic acid, including all *S.* Virchow's, one *S.* Java and *S.* Enteritidis pt21.
It is the second consecutive year that ESBL-positive *S.* Java strains were detected. Because it is a clone spreading in broilers in Germany and The Netherlands only, it indicates that these isolates were selected locally and not imported.

Twenty-seven gentamicin resistant strains, and seventeen neomycin resistant strains were found, which is an approximate increase of 100% compared to 2002. One ciprofloxacin resistant *S.* Kentucky strain (MIC > 4 µg/ml) was isolated from a human patient (in 2002 also a ciprofloxacin resistant *S* Kentucky was isolated from a human patient), and 271 (in 2002 168) flumequin/nalidixic acid resistant strains were found. These strains all showed reduced

susceptibility to ciprofloxacin (MIC ≥ 0,125 µg/ml). Fifteen fully susceptible *S.* Newport strains were found, one isolated from poultry and fourteen from human patients.

Table 3. Resistance percentages of the ten most prevalent *Salmonella* serotypes isolated in The Netherlands in 2003 (values for 2002 between brackets).

	Enteritidis (715)	Typhimurium (493)	Java (158)	Dublin (15)	Infantis (71)	Senftenberg (79)	Livingstone (35)	Derby (38)	Goldcoast (46)	Brandenburg (29)
Amoxicillin	5,6	41,8	51,3	0	9,9	1,3	2,9	5,3	15,2	3,4
Cefotaxime	0,3	0,4	1,3	0	0	0	0	0	0	0
Imipenem	0	0	0	0	0	0	0	0	0	0
Gentamicin	0,3	0,6	2,5	0	2,8	0	0	2,6	0	0
Neomycin	0,3	0,6	0	0	2,8	0	2,9	0	6,5	0
Doxy/tetracycline	2,5	57,0	22,8	0	14,1	17,7	2,9	39,5	10,9	31,0
Trim/sulpha	0,5	14,6	85,0	0	9,1	0	10,0	24,3	2,9	14,3
Sulphamethoxazole	5,7	47,4	33,3	-	12,5	18,8	40,0	-	33,3	-
Trimethoprim	0,6	13,4	98,1	0	7,1	1,3	20,0	26,3	6,5	13,8
Ciprofloxacin	0	0	0	0	0	0	0	0	0	0
Flum/Nalidixic acid	13,8(6,0)	2,6	34,2	0	9,9(2,2)	0	0	0	0	0
Chloramphenicol	0,6	25,4	1,9	20,0	0	8,9	2,9	5,3	2,2	0
Florfenicol	0,1	21,3	0	0	0	1,3	0	0	0	0
% fully Sensitive	78,7	34,3	1,9	73,3	73,2	79,7	77,1	55,3	82,6	62,1
% R to 1 ant.	19,2	21,9	23,4	26,7	12,7	11,4	5,7	15,8	2,2	27,6
% R to 2 ant.	1,0	12,8	41,8	0	8,5	6,3	14,3	21,1	2,2	10,3
% R to 3 ant.	0,7	7,1	22,2	0	2,8	1,3	0	7,9	8,7	0
% R to 4 ant.	0,3	16,4	10,8	0	2,8	0	0	0	4,3	0
% R to > 4 ant.	0,1	7,5	0	0	0	1,3	2,9	0	0	0

In Table 3 resistance percentages are presented for the most prevalent serotypes isolated in The Netherlands in 2003. The highest resistance levels are observed in *S.* Typhimurium and *S.* Java, the serotypes harbouring the highest percentage of multiple resistant isolates. Resistance to flumequin/nalidixic acid has increased in *S.* Enteritidis and *S.* Infantis, the latter isolated from poultry raw meat products (5) and human infections (2).
Multiple resistances to more than 2 antibiotics was predominantly observed in *S.* Typhimurium and *S.* Java.

S. Enteritidis

In Table 4 resistance percentages for *S.* Enteritidis and it most prevalent phagetypes are presented. In The Netherlands, human infections caused by *S.* Enteritidis are predominantly related to the consumption of raw shell eggs. The differences in resistance profile of strains from human infections and from layers/eggs are striking. In 2003 only one nalidixic acid resistant strain (Pt6) was isolated from an egg, while from human infections 85 Nal-resistant strains were isolated, predominantly Pt1. The relation between the increased imports of eggs as a result of the Avian Influenza epidemic in The Netherlands in 2003, and the increased incidence of human cases of salmonella infections in the summer of 2003 (estimated increase of 7500 cases) is described elsewhere (van Pelt *et al.* Eurosurveillance Monthly archives 2004, vol 9, Issue 7 - 8).

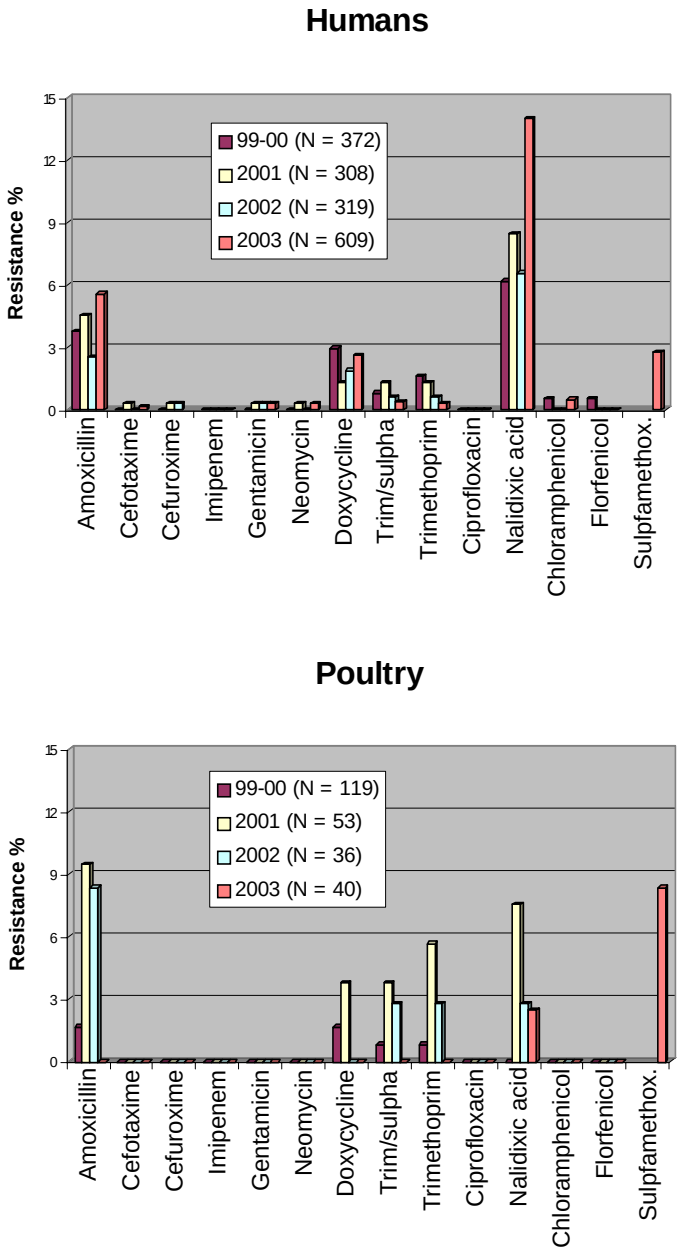
Table 4. Resistance percentages of *S.* Enteritidis and phagetypes 4, 21, 1, 6, 8, 14b, 7 and 6a isolated from different sources in 2003.

	Enteritidis			Phage types							
	Human (609)	**Layers/eggs (40)**	**Broiler/Meat (21)**	**Pt4 (199)**	**Pt21 (155)**	**Pt1 (104)**	**Pt6 (54)**	**Pt8 (76)**	**Pt14b (38)**	**Pt7 (11)**	**Pt6a (30)**
Amoxicillin	5,6	0	14,3	2,5	1,9	1,0	18,5	0	0	9,1	50,0
Cefotaxime	0,2	0	0	0,5	0,6	0	0	0	0	0	0
Imipenem	0	0	0	0	0	0	0	0	0	0	0
Gentamicin	0,3	0	0	0	0	1,0	0	0	0	0	3
Neomycin	0,3	0	0	0	0	0	0	0	0	0	3
Doxy/tetracycline	2,6	0	9,5	2,0	2,6	2,9	0	0	0	18,2	6,7
Trim/sulpha	0,4	0	6,3	0,6	1,6	0	0	0	0	0	0
Sulphamethoxazole	2,8	8,3	40,0	6,1	6,5	0	11,8	4,5	0	-	33,3
Trimethoprim	0,3	0	9,5	0,5	1,3	0	0	0	0	0	3,3
Ciprofloxacin	0	0	0	0	0	0	0	0	0	0	0
Flum/Nalidixic acid	14,0	2,5	19,0	4,5	7,7	52,9	9,3	1,3	5,3	27,3	16,7
Chloramphenicol	0,5	0	0	0,5	0,6	0	0	1,3	0	0	3,3
Florfenicol	0	0	0	0	0,6	0	0	0	0	0	0
% fully Sensitive	79	95	61,9	89,5	87,7	46,2	68,5	96,1	94,7	63,6	48,3
% R to 1 ant.	19	5	28,6	9,5	10,3	51,0	31,5	3,9	5,3	27,3	37,9
% R to 2 ant.	1,1	0	0	1	0	1,9	0	0	0	0	10,3
% R to 3 ant.	0,7	0	4,8	0	1,3	1	0	0	0	9,1	0
% R to 4 ant.	0	0	4,8	0	0,6	0	0	0	0	0	0
% R to > 4 ant.	0,2	0	0	0	0	0	0	0	0	0	3,4
% Human source	-	-	-	83	89	88	76	87	82	64	93

S. Enteritidis isolated from layers and eggs was susceptible to most antibiotics tested. The strains from broilers and meat products showed more resistances. However the number of strains examined was small and the source of the raw meat products was unknown. It is possible that (part of) these products were imported. Interestingly resistance profiles may be phagetype specific. Highest quinolone resistances was observed in Pt1. Multiple resistance is not very common in *S.* Enteritidis. Incidentally multiple resistant strains were observed in PT1, Pt6a, Pt7 and Pt21.

Trends in resistance are limited to flumequin/nalidixic acid resistance in human isolates (Fig. 1) and to a lesser extend amoxicillin resistance.

Figure 1. Trends in resistance percentages of *S.* Enteritidis isolated from humans and poultry (predominantly from layers, reproduction animals and poultry meat of undefined origin) from 1999 - 2002.



S. Typhimurium

Resistance percentages of *S.* Typhimurium in 2003 were strongly determined by the relatively large numbers of DT104 (28%) and Ft 507 (23%) included, being the predominant phage types of *S.* Typhimurium, both in food-animals and in humans, similar as in 2002 (Table 5). In 2003 thirteen flumequin/nalidixic acid resistant *S.* Typhimurium isolates were found, four were DT104, three were Ft 507, three Ft 60, and one Ft 3, 61 and 63, respectively. Ten of these strains were isolated from human patients, two (DT104 and Ft507) from poultry products and one (Ft3) from an unidentified bird species. All flumequin/nalidixic acid resistant isolates demonstrated reduced susceptibility to ciprofloxacin but were not high-level ciprofloxacin resistant.

Resistance levels and multiple resistances were substantially higher in *S.* Typhimurium than in *S.* Enteritidis (table 5, Fig. 2). Multiple resistance is greatly determined by the proportion of DT104, which is which is highest in cattle and poultry, however in these animals the number of strains examined were relatively small.

Trends in resistance in *S.* Typhimurium are difficult to determine in all sources (Fig. 2) because of the influence of the presence of multiple resistant clones and the relatively small number of isolates from cattle and poultry. Specifically when the total numbers of strains per year are relatively small the variability in the resistance percentages is high (eg. in poultry).

Table 5. Resistance percentages of S. Typhimurium and phage types DT104 and ft 507 isolated from different sources in 2003.

	***S.* Typhimurium**				**Phage types**	
	Human (346)	**Pigs (64)**	**Cattle (20)**	**Poultry (22)**	**DT104 (138)**	**ft507 (113)**
Amoxicillin	44,5	31,3	50,0	54,5	86,2	33,6
Cefotaxime	0,6	0	0	0	0	1
Imipenem	0	0	0	0	0	0
Gentamicin	0,9	0	0	0	0	1,8
Neomycin	0,6	0	0	0	0	0
Doxy/tetracycline	57,8	62,5	60,0	63,6	85,5	61,9
Trim/sulpha	13,2	22,8	14,3	18,8	9,3	21,8
Sulphamethoxazole	51,5	14,3	33,3	50,0	83,9	42,3
Trimethoprim	12,1	21,9	10,0	13,6	6,5	20,4
Ciprofloxacin	0	0	0	0	0	0
Flum/Nalidixic acid	2,9	0	0	9,1	2,9	2,7
Chloramphenicol	25,4	17,2	50,0	45,5	79,0	2,7
Florfenicol	21,4	14,1	50,0	36,4	72,5	0
% fully Sensitive	33,2	29,7	30	27,3	6,5	28,3
% R to 1 ant.	22,0	29,7	20	18,2	11,6	31,9
% R to 2 ant.	13,3	17,2	0	4,5	0,7	24,8
% R to 3 ant.	7,5	9,4	0	9,1	7,2	10,7
% R to 4 ant.	16,2	14,1	40	22,7	52,9	2,7
% R to > 4 ant.	7,8	0	10	18,2	21,0	1,8
DT104: %	28%	19%	50%	55%	70% human	79% human

Figure 2. Percentages of S. Typhimurium strains fully susceptible, resistant to one, two, three, four and more than four antibiotics in humans, pigs, cattle and poultry in The Netherlands in 2003.

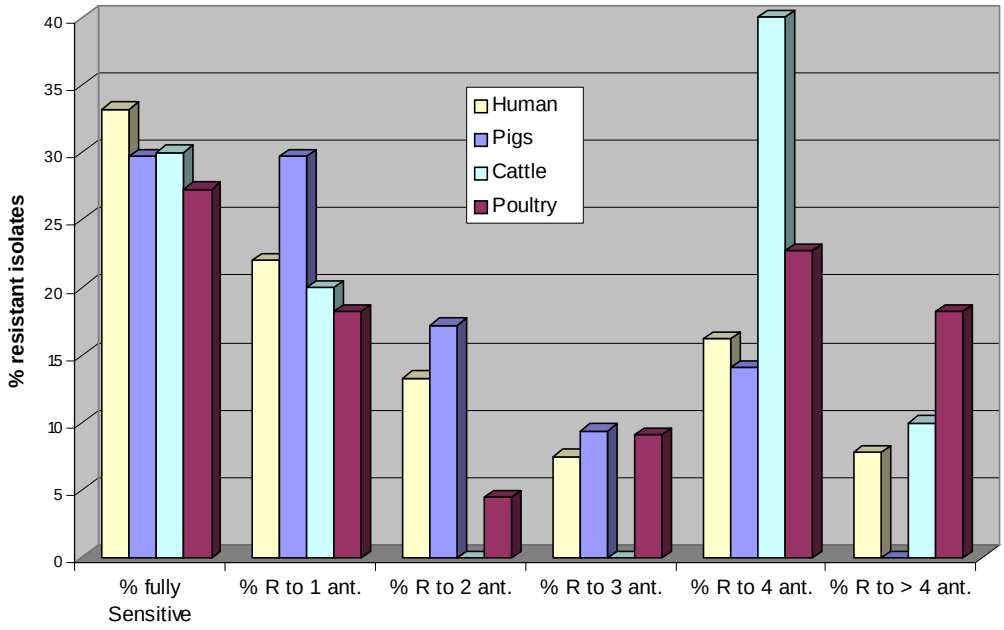
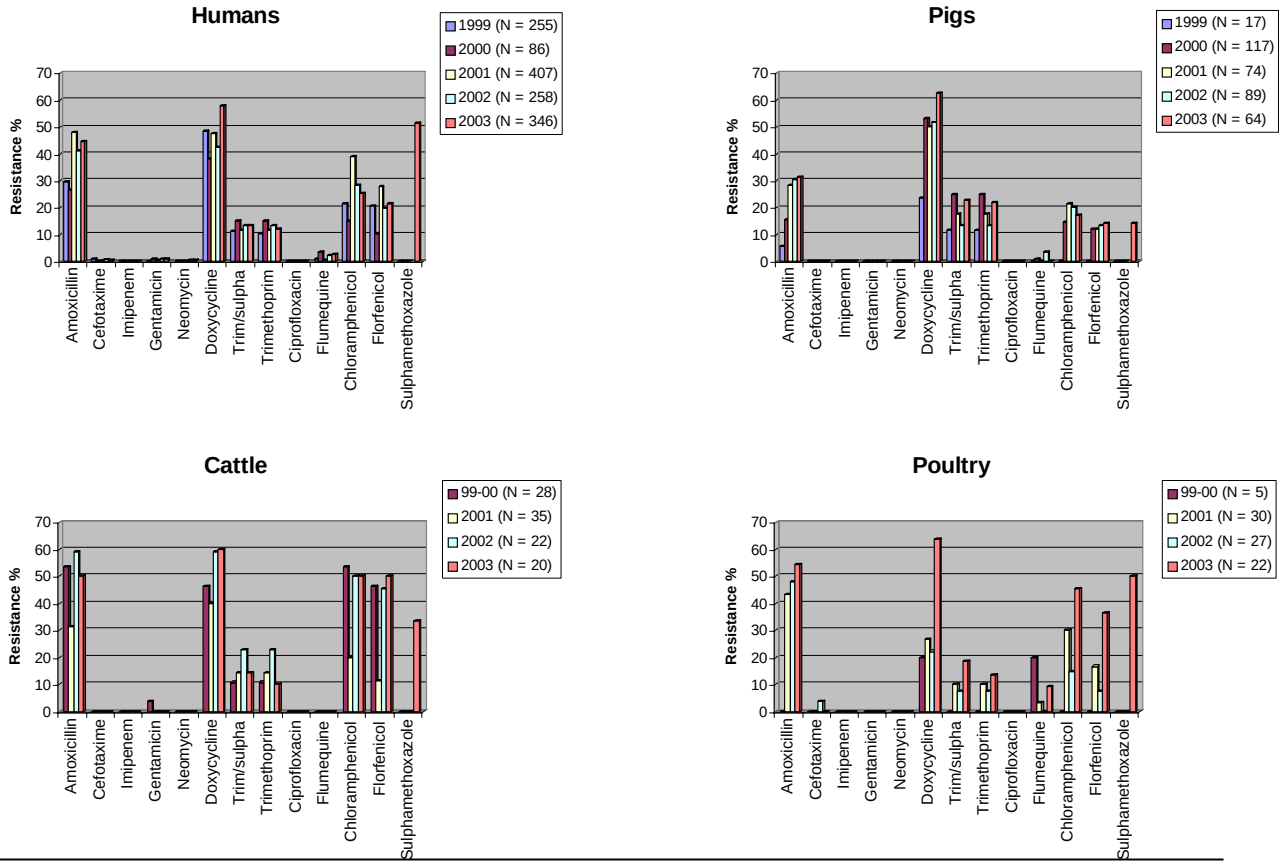


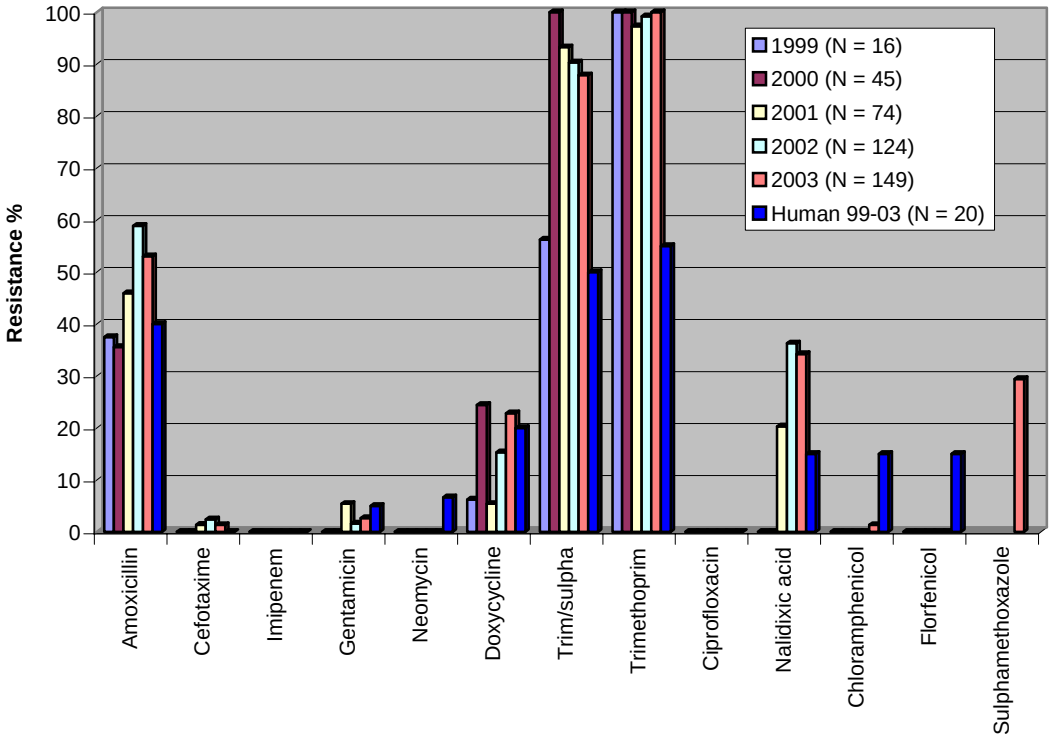
Figure 3. Trends in resistance percentages of *S.* Typhimurium isolated from humans and food-animals from 1999 - 2003



S. Paratyphi B var. Java

S. Java is the predominant serotype isolated from broilers since 1998. Clonal distribution in The Netherlands and Germany in broiler production of a strain 100% resistant to trimethoprim and increasingly resistant to flumequin is the cause. In 2003 only 2 strains were isolated from human patients and both strains were phenotypically different from the clone spreading in broilers. From poultry 149 strains were isolated, all harbouring the phenotype typical for the clone, four of these strains were isolated from layers, all others from broilers of broiler meat products. Flumequin/nalidixic acid resistance in *S.* Java isolated from poultry has remained stable in 2003 (Fig. 3). No ciprofloxacin resistant strains were found.

Figure 4. Trends in resistance percentages of *S.* Paratyphi B var. Java isolated from poultry from 1999 – 2003 and humans (blue bars indicate all humans isolates from 1999 – 2003 (N = 20))

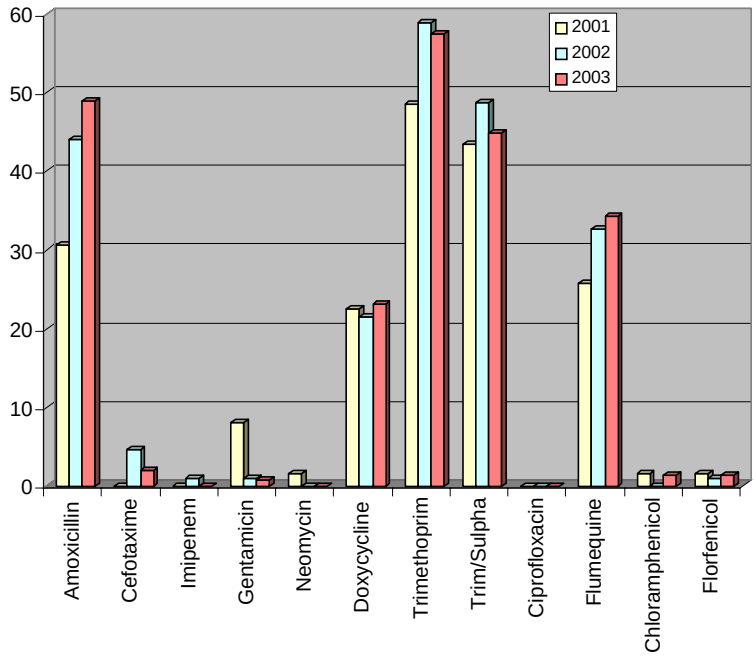


Salmonella spp. in raw meat products of food-animals

Tabel 6. Resistance % of *Salmonella* spp. isolated from raw meat from poultry, beef and pork products in 2003

	Poultry N = 143	Beef N = 13	Pork N = 15
Amoxicilline	48,9	15,4	13,3
Cefotaxime	2,1	0	0
Imipenem	0	0	0
Gentamicin	0,7	0	0
Neomycin	0	0	0
Doxycycline	23.1	23,1	26,7
Trim/suplha	44,8	15,4	20,0
Trimethoprim	57,3	15,4	20,0
Ciprofloxacin	0	0	0
Flumequin	34,3	0	0
Chloramphenicol	1,4	15,4	6,7
Florfenicol	1,4	7,7	6,7

Figure 5. Trends in resistance % of *Salmonella* spp. isolated from chicken products in the Netherlands in 2001 (N = 62), 2002 (N = 107) and 2003 (N = 143)



In general the resistance levels are highest in poultry products compared to beef and pork (table 6). The observed resistance patterns and trends in the chicken isolates are strongly determined by the large contribution of *S.* Java (table 7). In beef and pork resistance is limited to older drug classes, while only in poultry products resistance to third-generation cefalosporins (cefotaxime), gentamicin and the quinolones occurs. Resistance trends are only presented for poultry products because in beef and pork the numbers of isolates examined are too small to provide an accurate estimate. In poultry products resistance to amoxicillin and flumequin increased from 2001 to 2003.

Table 7. Distribution of Salmonella serotypes, in poultry meat at retail (Surveillance data of Food and Consumer Product Safety Authority (VWA-KvW))

	1997	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**
Poultry meat sample size at retail	1314	1077	859	1454	1578	1600	1510
Salmonella spp. positive (%)	29.1	20.2	17.6	21	16.3	13.4	11.3
Enteritidis positive (%)	8,8	nd	4,8	1,4	1,4	0,3	1.0
Paratyphi B var. Java positive (%)	4.4	2.3	2.4	7.0	7.0	7.2	5.1
Main serotypes 1997-2003 As a fraction of all isolates (%)							
Paratyphi B var. Java	15	11.4	13.9	33.1	43.2	53.5	45.6
Enteritidis	20.2	12.8	26.4	6.6	8.2	2.3	8.8
Hadar	10.1	6.1	4.5	3.3	4.2	0.9	1.8
Indiana	6.1	8.3	9.3	10.2	11.6	6.5	6.4
Infantis	9.2	5	3.6	6.6	7	7.9	11.7
Virchow	4.6	2.8	2.6	10.2	3.5	5.6	5.8
Typhimurium (DT104)	7.8	3.6(1.8)	1.3(0.7)	0.1(0.1)	7.4(7)	7.4(2.8)	5.8(5.3)
Other types	27	50	38.4	29.9	14.9	15.8	5.8

Salmonella spp. in animal feeds

In Table 8 resistance data are presented for salmonella's isolated from animal feeds. In 2003 1570 *Salmonella* spp. were sent to RIVM for sero-, and phage typing, and 481 of these strains were tested for susceptibility at CIDC Lelystad. A wide variety of serotypes (N = 106) were isolated, *S.* Agona, *S.* Lexington, *S.* Senftenberg, *S.* Mbandaka, *S.* Cubana and *S.* Rissen were the most prevalent ones. Included were 37 *S.* Enteritidis isolates of various phage types, 7 *S.* Typhimurium (1 DT104, 2 Ft 507), and 6 *S.* Java isolates.

The resistance percentages were much lower than those for the human and food-animal isolates. The data show that animal feeds may be a source for introduction of important *Salmonella* sero-, or phage types in food animals, the contribution to the resistance levels in the predominant serotypes seems to be small.

Table 8. Numbers of isolates of *Salmonella* spp. per single and or compound feed type tested for susceptibility and resistance percentages (R%), in 2001 and 2002 combined and 2003.

Feed source (N)	Fishmeal (10)	Animal meal (15)	Other single feed (104)	Other composite feed (1)	Soy beans toasted (90)	Soybean expeller/extracted/hulls (93)	Canola/rapeseedexpeller/extracted (89)	Cow feed meal/piece (6)	Pig feed piece/meal (13)	Chicken feed(layers) meal/piece (7)	Chicken feed(broilers) meal/piece (5)	Dog food meal/piece (5)	Other feed meal/piece (43)	2003 (N=481)	2001-2002 (N=340)
Amoxicillin	0	1	0	0	1	0	0	0	0	0	0	0	2	0,8%	2,1%
Cefotaxime	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0%	0,0%
Cefuroxim	0	1	1	0	1	0	0	0	0	0	0	0	0	1,3%	0,0%
Imipenem	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0%	0,0%
Gentamicin	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0%	0,0%
Neomycin	0	0	1	0	0	0	0	0	0	0	0	0	0	0,2%	0,0%
Tetracycline	0	2	9	0	5	3	1	1	2	1	0	0	8	6,7%	3,8%
Trim/sulpha	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0%	0,6%
Trimethoprim	0	1	1	0	0	1	1	0	0	1	0	0	1	1,2%	0,6%
Ciprofloxacin	0	0	0	0	0	0	0	0	0	0	0	0	0	0,0%	0,0%
Nalidixic acid	0	1	1	0	0	0	0	0	0	0	0	0	0	0,4%	1,5%
Chloramphenicol	0	1	2	0	1	0	0	0	0	0	0	0	2	1,2%	2,1%
Florfenicol	0	0	0	0	1	0	0	0	0	0	0	0	1	0,4%	1,2%

Campylobacter spp.

Table 9. MIC distribution (in %) for *C. jejuni* isolated from broilers (N =48) and *C. coli* isolated from slaughter pigs (N = 193) in The Netherlands in 2003

2003	MIC distribution (µg/ml)														
	0,125	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**R%**
Amoxicillin		0,8	3,3	16,1	21,1	21,1	19,8	2,1	2,1	13,6					15,7
Gentamicin		48,3	49,6	2,1											0
Neomycin			18,6	71,9	7,9			1,2	0,4						1,7
Streptomycin				19,8	13,2	3,3	0,4	5,4	31,0	8,3	3,3	15,3			63,2
Doxycycline	16,1	3,7	8,7	1,2	2,1	7,4	23,1	26,4	11,2						60,7
Trim/sulpha		2,1	14,9	14,0	11,2	11,2	3,3	24,8	18,2	0,4					43,4
Sulphamethoxazole							11,6	16,9	9,9	13,6	3,7	19,8	23,1	1,2	24,4
Ciprofloxacin	57,4	26,4	3,7	0,4			1,7	4,5	5,8						12,0
Nalidixic acid					9,9	43,4	31,8	2,9		0,4	7,0	4,5			12,0
Erythromycin			2,5	7,0	27,3	36,0	15,7	0,4			11,2				11,6
Metronidazole			5,0	17,8	14,9	13,2	7,0	7,0	9,9	17,4	7,9				49,2
Chloramphenicol					19,4	52,9	23,1	4,5							0

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

Table 9 presents the MIC-distributions and resistance percentages for all campylobacters isolated from broilers and slaughter pigs in 2003. In Table 10 these resistance percentages are presented separately for both animal and *Campylobacter* species and in Figure 5 the trends in resistance from 1999 – 2002 are presented.
Highest resistance percentages can be observed for streptomycin, doxycycline, metronidazole and (potentiated) sulphonamides. Resistance to the quinolones, erythromycin and amixicillin are substantial. However differences in level of resistance exist both between *C. jejuni* and *C. coli*, and between pigs and broilers. Figure 5 shows that *C. coli* from poultry generally shows higher resistance levels that *C. jejuni* from poultry, as a result of the differences in species specific capacity to become resistant. Table 9 shows that resistance levels for streptomycin, doxycycline and (potentiated) sulphonamides are highest in pigs while those for amoxicillin, the quinolones and metronidazole are highest in poultry. Resistance to erythromycine is only present in *C. coli* from pigs. Campylobacter is the only bacterial species involved in the Dutch monitoring programme in which the prevalence of multiple resistant strains is highest in pigs compared to poultry. This may reflect the difference between *C. jejuni* and *C. coli* in their capacity to become resistant (fig. 7).

Trends in resistance can be observed in poultry, in spite of control measures taken and active propagation of prudent use policies in The Netherlands, resistance to ciprofloxacin still slightly increases (fig. 6).

Table 10. Resistance percentages of *C. jejuni* and *C. coli* isolated from broilers and slaughter pigs in 2003

***Campylobacter* spp. 2003**	**Broilers**	**Pigs**
	***C. jejuni* (N = 48)**	***C. coli* (N = 193)**
Amoxicillin	33,3	11,4
Gentamicin	0	0
Neomycin	8,3	0
Streptomycin	0	79,3
Doxycycline	33,3	67,9
Trim/sulpha	2,1	53,9
Sulphamethoxazole	2,1	30,1
Ciprofloxacin	41,7	4,7
Nalidixic acid	41,7	4,7
Erythromycin	0	14,5
Metronidazole	64,6	28,0
Chloramphenicol	0	0
% fully Sensitive	18,8	3,1
% R to 1 ant.	25,0	9,3
% R to 2 ant.	29,2	20,7
% R to 3 ant.	8,3	29,5
% R to 4 ant.	16,7	22,3
% R to > 4 ant.	2,1	15,0

Figure 6. Trends in resistance percentages of *C. coli* isolated from slaughter pigs and broilers, and *C. jejuni* isolated from broilers from 1999 - 2002

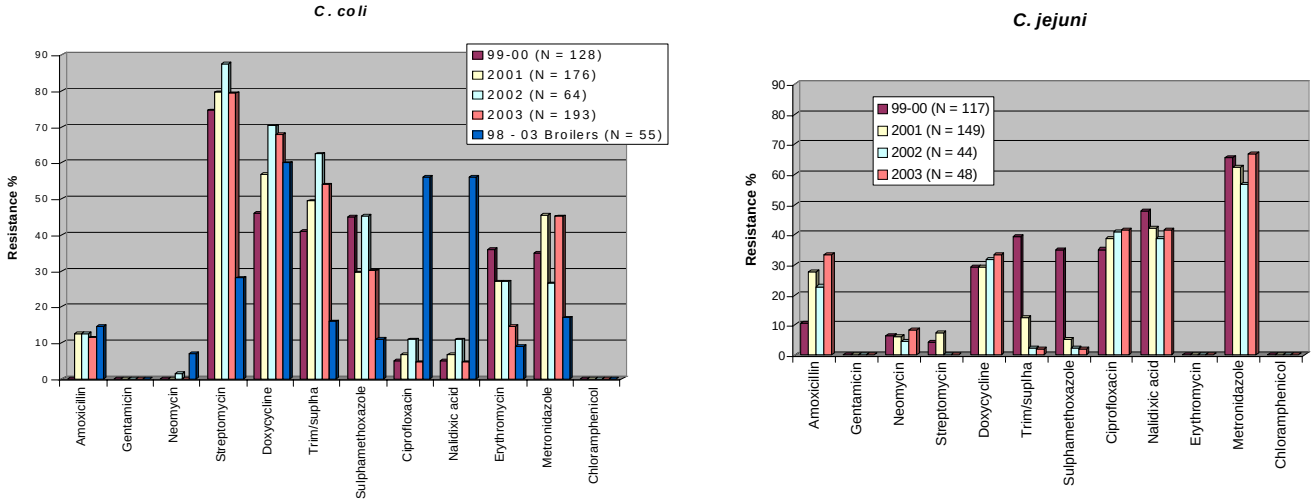


Figure 7. Percentages of *Campylobacter* strains fully susceptible, resistant to one, two, three, four and more than four antibiotics in pigs and poultry in The Netherlands in 2003.

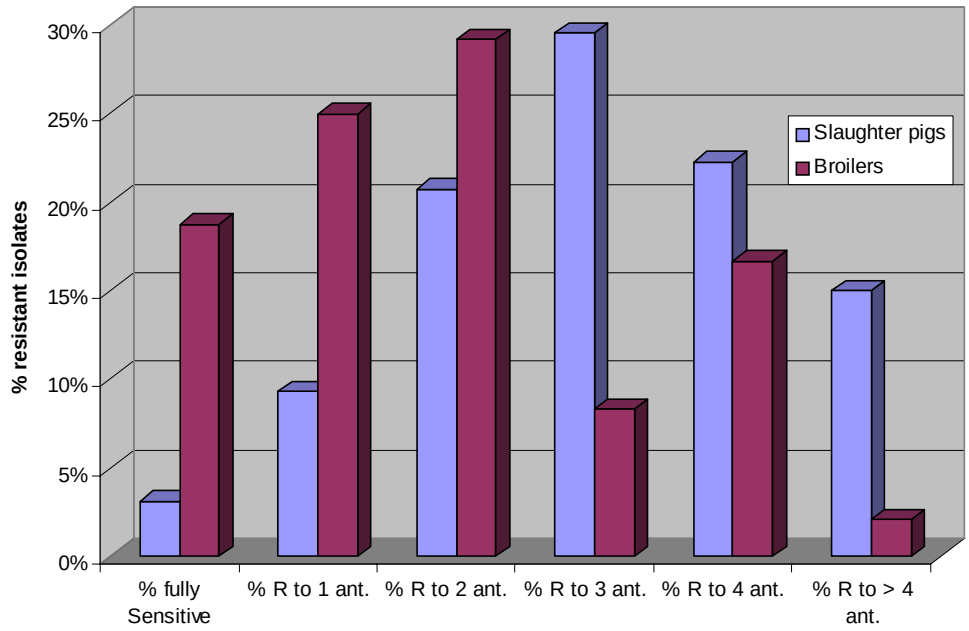
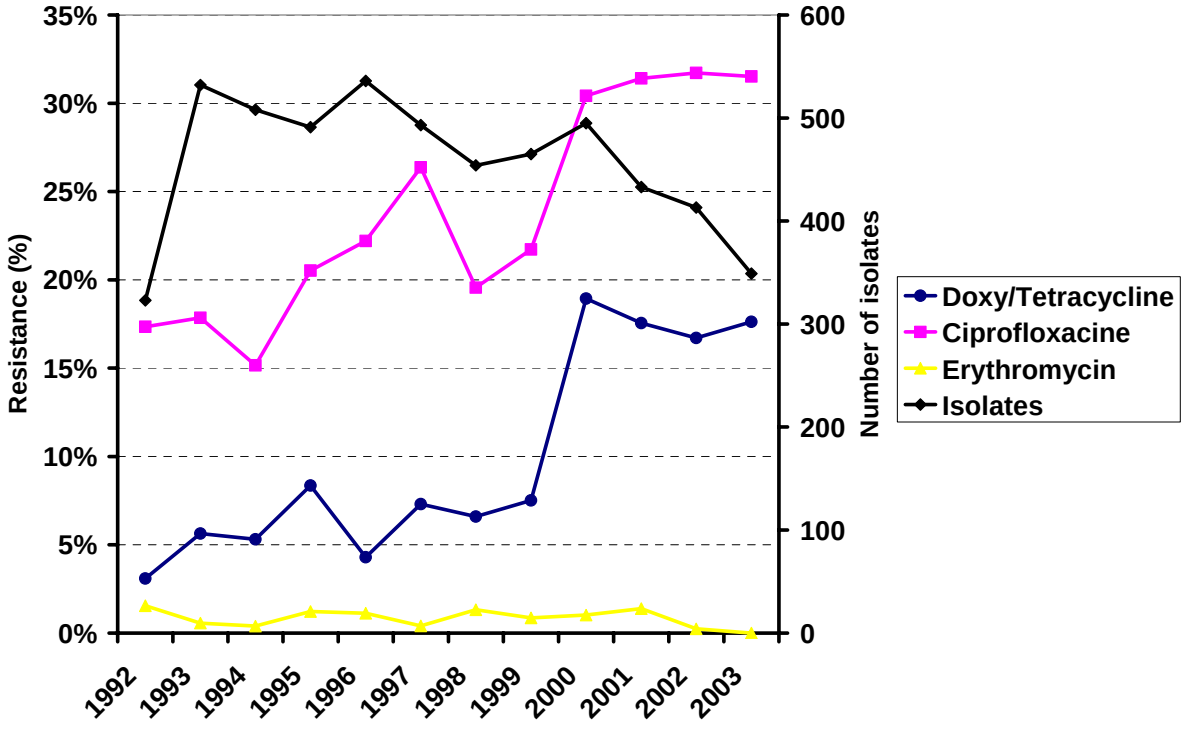


Figure 8 shows that in human *Campylobacter* spp. resistance to fluoroquinolones (data are based on disk diffusion tests for norfloxacin, ofloxacin and ciprofloxacin) slowly increased in the last decade, but remained stable around 31% since 2000. In 2000 both resistance to fluoroquinolones and tetracyclines increased suddenly approximately 10%. A biological

Figure 8. Trends in resistance % of *Campylobacter* spp. isolated from humans isolated between 1993 and 2003 at the regional Public Health Laboratories (PHLs) of Arnhem and Heerlen covering 990.000 inhabitants



explanation for this phenomenon does not exist. After 2000 the levels remained stable at approximately 31%. Resistance to macrolides remained stable at a very low level and was not detected in 2003.

Shigella toxin producing *E. coli* O157

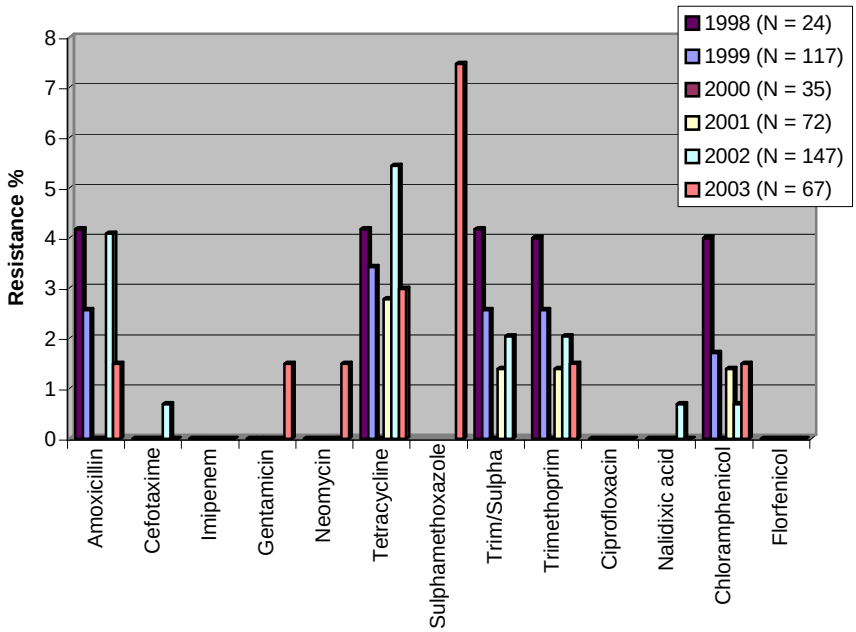
In 2003 67 strains of *E. coli* O157 were sent to RIVM for typing purposes or isolated from specimens taken from human faeces (64), veal calves (1) and dairy cattle (2) in an attempt to trace a human clinical infection. Table 11 demonstrates that in general the resistance levels are low. One isolate was multiple resistant to gentamicin, neomycin, tetracycline, trimethoprim, sulphamethoxazole and chloramphenicol, indicating the presence of an integron. No ESBL-positive isolates were detected in 2003. Trends in resistance cannot be observed, resistance is still limited to incidentally occurring individual strains.

Table 11. MIC distribution (in %) for *E. coli* O157 (N = 67) isolated in The Netherlands in 2003 from human faeces and cattle (N = 3)

	MIC distribution (µg/ml)															
2003	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**R%**
Amoxicillin								3,0	94,0	1,5			1,5				1,5
Cefotaxim				98,5	1,5												0
Imipenem				85,1	11,9	3,0											0
Gentamicin					14,9	73,1	7,5	3,0					1,5				1,5
Neomycin							89,6	6,0	3,0						1,5		1,5
Tetracycline							1,5	94,0	1,5					3,0			3,0
Sulphamethoxazole										92,5						7,5	7,5
Trimethoprim						97,0	1,5							1,5			1,5
Ciprofloxacin			98,5	1,5													0
Nalidixic acid								29,9	68,7	1,5							0
Chloramphenicol									6,0	89,6	3,0				1,5		1,5
Florfenicol									41,8	58,2	0,0						0

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

Figure 9. Trends in resistance percentages of *E. coli* O157 isolated in The Netherlands from 1998 - 2003



Food-borne commensal organisms

The level of antimicrobial resistance in randomly sampled commensal organisms of the intestinal tract directly reflects the selection pressure as a result of the use of antibiotics as therapeutics or growth promoters in animals, especially over time. For this purpose, *E. coli* and *Enterococcus faecium*, as indicator organisms for the Gram-negative and Gram-positive flora, are monitored. Isolation of bacteria from the intestine of randomly picked animals at slaughter aims to detect the development of resistance at the bacterial population level in food animals. Resistance percentages in tables 10 and 12 indicate the level of resistance in all *E. coli* and *E. faecium* strains of slaughter pigs and broilers, respectively. This method is inherently insensitive for detecting resistance. If resistance is detected, even at low percentages, it indicates that the number of animals or groups of animals that carry these resistant bacteria is still substantial

Escherichia coli

Both in slaughter pigs and broilers, the older classes of antibiotics, amoxicillin, doxycycline, (potentiated) trimethoprim and chloramphenicol showed the highest resistance levels (Table 12). Moreover, the resistance levels in broilers except those for florfenicol, are always higher than those in pigs. The resistance levels show a tendency to increase in pigs only (Fig. 9). The increase observed for the older classes of antibiotics in pigs indicates an increase in the presence of class-I integrons encoding for multiple-resistance, which are known to be highly prevalent in commensal *E. coli*'s from food-animals in The Netherlands. In the presence of integrons co-selection will be very important in the evolution of resistance and will result in the continuous presence of certain resistance genes, even without selection pressure by usage of the specific antibiotics.
In broilers resistance to flumequine was very substantial (34,5%), but slightly lower than in 2002. Flumequin resistance levels seem to vary around 35% in broilers, real trends cannot be observed. In broilers five of these strains were also ciprofloxacin resistant.
In broilers four cefotaxime resistant strains were isolated that were suspected to be ESBL-positive, and in pigs one.
Figure 8 shows that in broilers (46,1%) multiple resistance to three or more antibiotics was substantially higher than in pigs (26,1%). This may reflect difference in use patterns of antibiotics in these animals but may also be caused by the husbandry systems. Broiler fattening takes approximately six weeks, while pig fattening takes about six months. Therefore in pigs after selection of resistance before and during weaning, a reduction of resistance can occur during the months of fattening. In Sweden multiple resistance is much less common, the levels are 15% in pigs and 3% in chickens (SVARM 2002 and 2003).

Table 12. MIC distributions (in %) for *E. coli* isolated from slaughter pigs (N = 155) and broilers (N = 165) in The Netherlands in 2003.

	MIC distribution (µg/ml)																
Slaughter Pigs	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**R%**
Amoxicillin						1,3	1,3	22,6	41,3	5,8			27,7				27,7
Cefotaxim				98,1	1,3			0,6									0,6
Imipenem				96,1	3,9												0
Gentamicin					17,4	58,7	17,4	4,5	0,6		0,6	0,6					1,3
Neomycin							78,1	12,9	4,5	0,6	0,6	0,6	0,6	1,3	0,6		3,2
Tetracycline							3,9	18,7	7,7	0,6	0,6		29,0	39,4			69,7
Trim/sulpha					51,6	3,9	0,6	0,6	0,6				42,6				43,2
Trimethoprim						51,0	3,9	0,6	0,6					43,9			43,9
Ciprofloxacin			100														0
Flumequin						69,7	30,3										0
Chloramphenicol									17,4	69,7	5,2	3,9	1,9		1,9		7,7
Florfenicol								2,6	32,9	61,3	2,6	0,6					0,6
	MIC distribution (µg/ml)																
Broilers	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**R%**
Amoxicillin							1,2	13,9	32,7	2,4			49,7				49,7
Cefotaxim				96,4	0,6		0,6	1,2		0,6	0,6						2,4
Imipenem				95,2	4,8												0
Gentamicin					11,5	63,0	17,6	4,8			1,2	1,2	0,6				3,0
Neomycin							73,9	9,7	1,2	1,2	2,4	5,5	3,6	0,6	1,8		11,5
Tetracycline							9,7	21,8	8,5			1,2	23,0	35,8			60,0
Trim/sulpha					45,5	7,3	1,2						46,1				46,1
Trimethoprim						44,8	9,1							46,1			46,1
Ciprofloxacin			64,2	6,1	20,0	6,1	0,6		1,8	0,6	0,6						3,0
Flumequin						43,0	20,0		2,4	13,9	15,8	0,6	4,2				34,5
Chloramphenicol									22,4	53,9	7,9	2,4	1,8	0,6	10,9		15,8
Florfenicol								0,6	38,8	53,3	7,3						0

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

Figure 10. Percentages of *E, coli* strains fully susceptible, resistant to one, two, three, four and more than four antibiotics in pigs and poultry in The Netherlands in 2003.

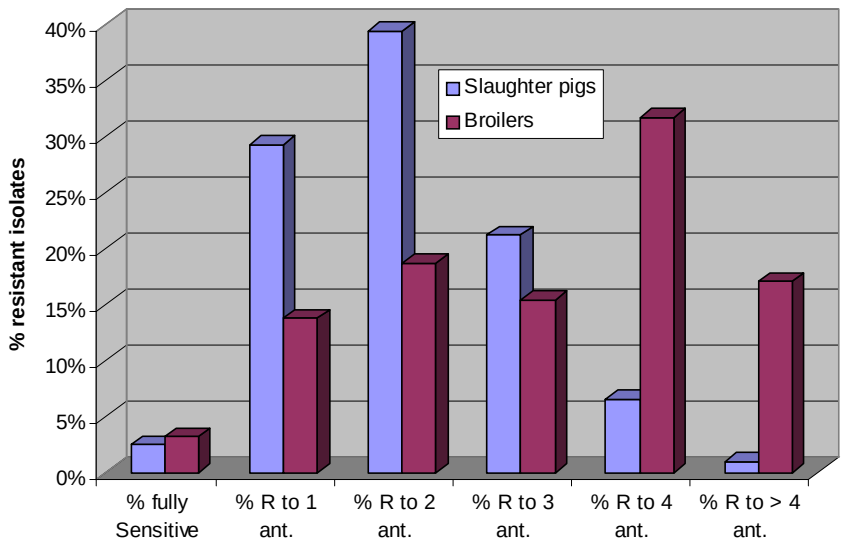
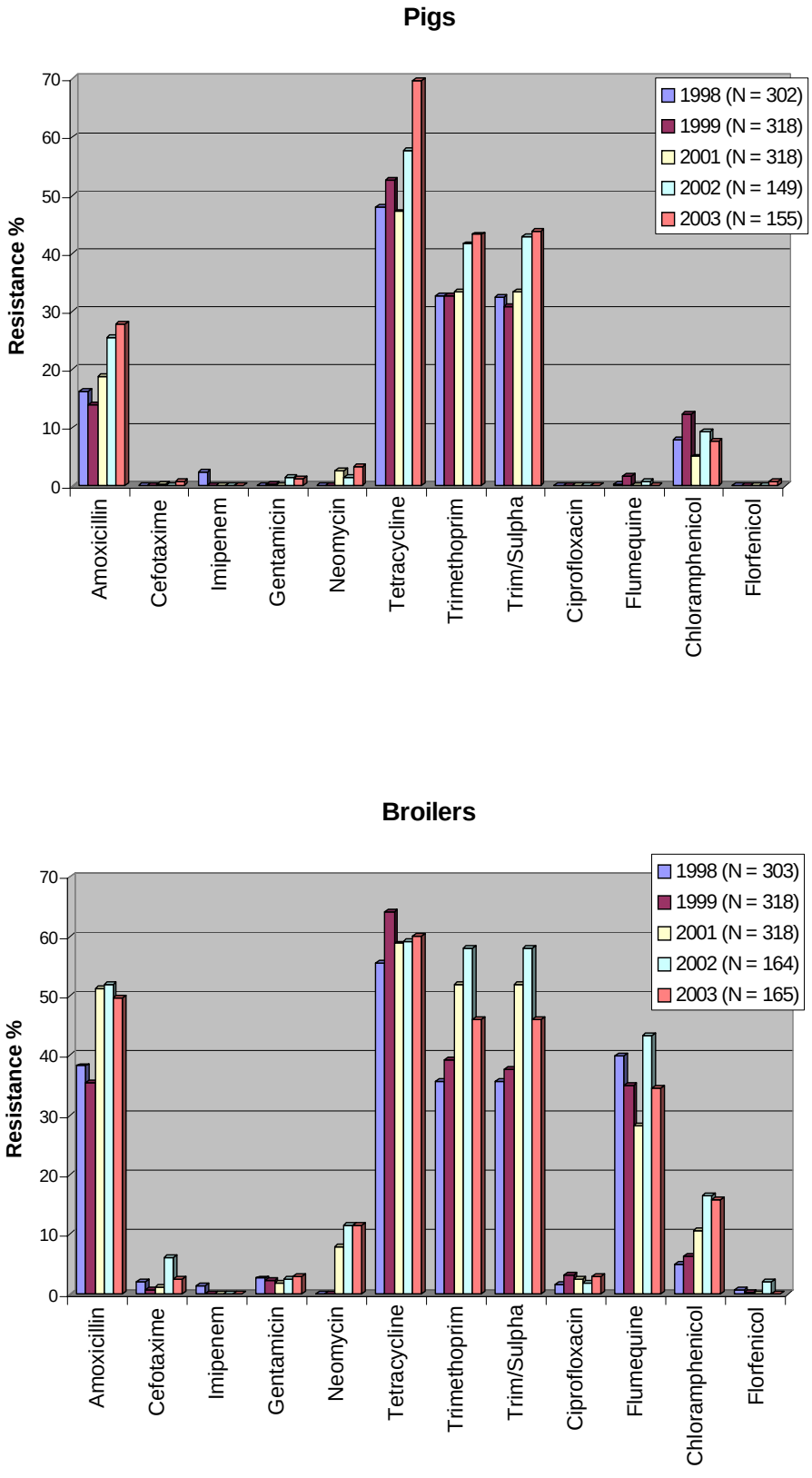


Figure11. Trends in resistance percentages of *E. coli* isolated from slaughter pigs and broilers in The Netherlands from 1998 - 2003



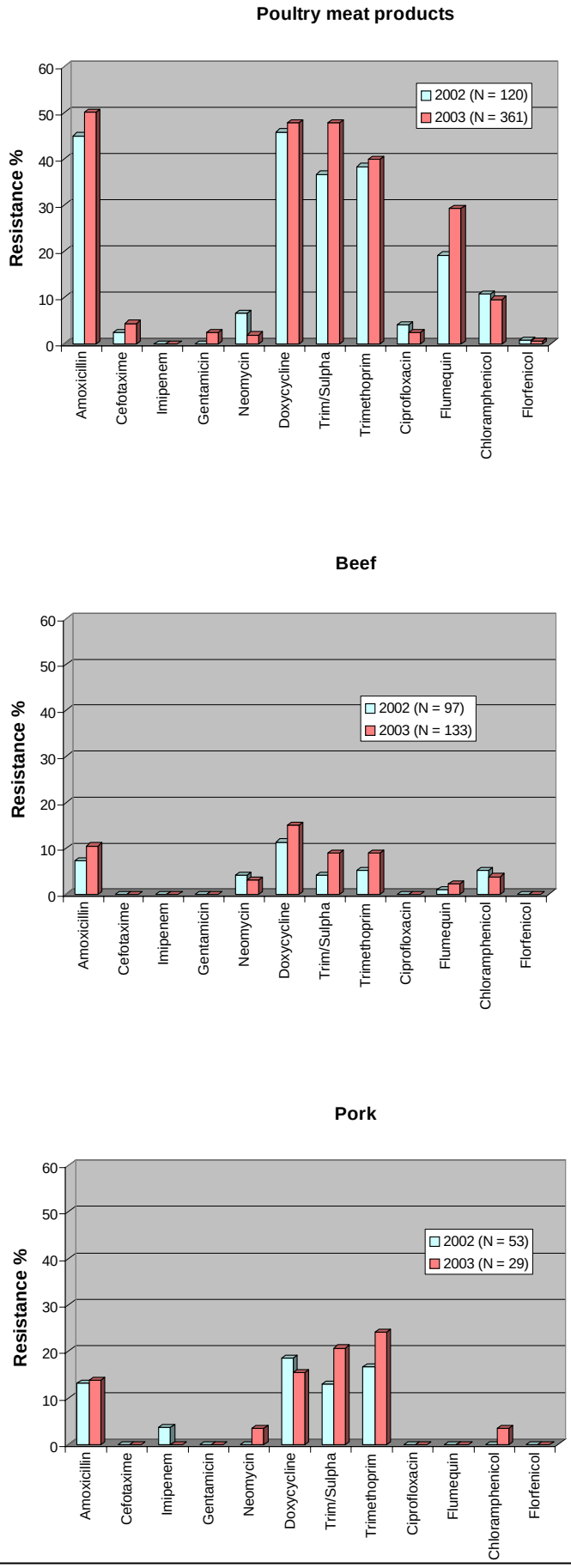
E. coli in raw meat products of food-animals

Table 13. Resistance % of *E. coli* isolated from raw meat products of poultry, beef and pork in The Netherlands in 2003

	Poultry N = 361	Beef N = 153	Pork N = 29
Amoxicillin	50,1	10,5	13,8
Cefotaxime	4,4	0	0
Imipenem	0	0	0
Gentamicin	2,5	0	0
Neomycin	1,9	3,0	3,4
Doxycycline	47,8	15,0	15,4
Trim/Sulpha	38,5	9,0	20,7
Trimethoprim	39,9	9,0	24,1
Ciprofloxacin	2,5	0	0,0
Flumequin	29,3	2,3	0,0
Chloramphenicol	9,6	3,8	3,4
Florfenicol	0,6	0,0	0,0
% fully Susceptible	30,1	69	82,7
% R to 1 ant.	18,8	10,3	5,3
% R to 2 ant.	16	10,3	2,3
% R to 3 ant.	19,9	10,3	6,8
% R to 4 ant.	11,6	0	2,3
% R to > 4 ant.	3,6	0	0,8

Resistance percentages of *E. coli* isolates from poultry products are very similar to those of isolates from broilers at slaughter, indicating that faecal contamination of poultry carcasses is an important factor in the transmission of *E. coli*. The proportion of fully susceptible strains is higher in meat products (table 13). In *E. coli* from beef, resistance percentages are similar to those in *E. coli* from pork, resistance is poultry products is at a substantially higher level. In strains isolated from pork, resistance percentages were lower than those found at slaughter. Resistance to flumequin was high in strains from poultry products, low in beef and not present in pork, reflecting the differences in use patterns of quinolones in these animals.
The resistance patterns: amoxicillin/trimethoprim/sulphonamides/chloramphenicol indicates the common presence of integrons, genetic structures involved in transmission of multiple resistance between *Enterobacteriaceae* of animal origin.
Figure 10 shows trends in resistances in the different meat products. Although the resistance percentages show a general tendency to increase, these data have to be interpreted carefully. The observed tendency may be a normal variation due to sampling methods used and not reflect a true increase.

Figure 12. Trends in resistance percentages of *E. coli* isolated from raw meat products of poultry, cattle and pigs in The Netherlands in 2003.



Enterococcus faecium, Enterococcus faecalis

In *E. faecium* isolated from broilers and pigs next to doxycycline, the highest resistance percentages were found for those antibiotics representing the growth promoters: bacitracin, flavomycin (intrinsic resistance in *E. faecium*), avilamycin in broilers, erythromycin and tilmicosin (macrolides), virginiamycin and quinupristin/dalfopristin (Synercid®) (streptogramins) in broilers and salinomycin (ionophore) (Table 14). Resistance to the glycopeptides vancomycin and teicoplanin is low (1,5 and 1,6%, respectively) and shows a tendency to further decrease. No amoxicillin or ciprofloxacin resistant strains were detected. High-level streptomycin resistant strains were present in both animal species but in a higher percentage in broilers.

Both in slaughter pigs and in broilers the resistance levels remained stable in 2003 compared to 2002 (Fig. 11).

For salinomycin in 2003 the MIC breakpoint was changed from 16 µg/ml as used in DANMAP to 4 µg/ml. In the Netherlands salinomycin is used intensively as coccidiostat in broilers and as growth promoter in slaughter pigs. Fig 13 shows that throughout the years the MIC-distributions of salinomycin are bimodal, showing a susceptible population with MICs varying from 1 – 4 µg/ml and a decreased susceptible population with MICs varying from 4 – 64 µg/ml. Until 2002 using 16 µg/ml as breakpoint the determined resistance levels (0 – 14%) were an underestimation of the true occurrence of resistance to salinomycin present in *E. faecium.* Interestingly in DANMAP 2002 in enterococci from broilers the MIC distributions are similar to those in figure 13. Indicating that in *E. faecium* strains from Danish broilers using 4 µg/ml as breakpoint, the resistance percentage would be 79.4% instead of 0% as published in DANMAP 2002. In Sweden narasin is tested representing the ionophores with 2 µg/ml as breakpoint and similar resistance levels in strains from broilers (78% in 2002) as found as in the Netherlands. In strains from pigs resistance to salinomycin increased from 35% in 1998 to 59% in 2003 indicating an increased use after the ban of the antimicrobial growth promoters in 1999. In strains from broilers after an initial decrease in resistance in 1999 and 200, the levels are stable at approximately 60%.

Multiple resistance to three or more antibiotics is much more common in *E. faecium* strains from broilers (64,2%) than in strains from pigs (28,8%) (Fig. 14). This is almost three time higher in broilers and seven times higher in pigs than in Swedish strains (SVARM 2002 and 2003).

Table 14. MIC distributions (In %) for *E. faecium* isolated from slaughter pigs (N = 198) and broilers (N = 123) in The Netherlands in 2003.

	MIC distribution (µg/ml)																
Slaughter Pigs	**0,015**	**0,03**	**0,06**	**0,12**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**R %**
Amoxicillin			0,5	0,5	2,0	7,6	25,3	30,3	29,8	3,5	0,5							0
Amox/clav. acid			0,5	0,5	2,5	7,1	25,8	30,3	30,8	2,5								0
Chloramphenicol									2,5	80,3	15,2	2,0						0
Doxycycline				7,1	8,1	0,5	0,5		0,5	5,6	45,5	29,8	2,5					83,3
Erythromycin				1,0	2,5	1,0	1,5	45,5	12,6	3,0			1,0	31,8				35,9
Vancomycin						86,4	9,1	2,0	0,5	0,5					1,5			1,5
Teicoplanin				3,0	46,0	37,4	11,6	0,5				0,5	1,0					1,5
Strep > 2000								4,5	> 2000									4,5
Genta > 500								0	> 500									0
Ciprofloxacin					2,5	25,3	40,9	23,7	6,1	1,5								0
Nitrofurantoin												1,0	25,3	71,2	2,5			2,5
Avilamycin								4,0	66,2	22,2	0,5	1,0	0,5	5,6				7,1
Bacitracin									4,0	26,3	5,1	1,0	3,5	9,6	50,5			50,5
Flavomycin															0,5	0,5	99,0	100
Salinomycin						1,5	12,1	23,7	3,5	43,9	15,2							59,1
Quinu/dalfopristin					1,0	3,5	1,0	60,6	30,3	3,0	0,5							3,5
Tilmicosin										2,0	47,5	16,7	1,5	0,5			31,8	33,8
Virginiamycin					3,0	1,0	35,9	48,5	5,6	3,5	1,5	0,5	0,5					2,5
	MIC distribution (µg/ml)																	
Broilers	**0,015**	**0,03**	**0,06**	**0,12**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**R %**
Amoxicillin				0,8	6,5	13,8	20,3	25,2	29,3	3,3	0,8							0
Amox/clav. acid				0,8	6,5	15,4	20,3	23,6	29,3	3,3	0,8							0
Chloramphenicol								0,8	4,9	61,0	26,8	6,5						0
Doxycycline			0,8	15,4	8,9		3,3	1,6	0,8	20,3	22,8	26,0						69,1
Erythromycin		1,6	8,9	5,7	1,6	7,3	8,9	3,3	0,8	1,6	3,3			56,9				62,6
Vancomycin						75,6	17,9	4,9							1,6			1,6
Teicoplanin				1,6	22,8	54,5	18,7	0,8			0,8		0,8					0,8
Strep > 2000								16,3	> 2000									16,3
Genta > 500								0,1	> 500									0,1
Ciprofloxacin						4,1	25,2	36,6	30,1	4,1								0
Nitrofurantoin										0,8	2,4	12,2	23,6	43,1	17,9			17,9
Avilamycin						1,6	2,4	2,4	19,5	25,2	4,1	1,6	4,9	38,2				44,7
Bacitracin								0,8	0,8	2,4	4,1	4,9	4,1	4,1	78,9			78,9
Flavomycin										0,8			0,8		2,4	4,9	91,1	99,2
Salinomycin						4,1	10,6	9,8	10,6	61,0	3,3				0,8			65,1
Quinu/dalfopristin					0,8	13,8	9,8	22,0	29,3	10,6	5,7	8,1						24,4
Tilmicosin								0,8	2,4	3,3	23,6	13,8	0,8	0,8			54,5	56,1
Virginiamycin					4,9	14,6	13,8	28,5	6,5	9,8	8,1	3,3	10,6					22,0

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

Figure 13. Trends in resistance percentages of *E. faecium* isolated from slaughter pigs and broilers in The Netherlands from 1998 - 2003

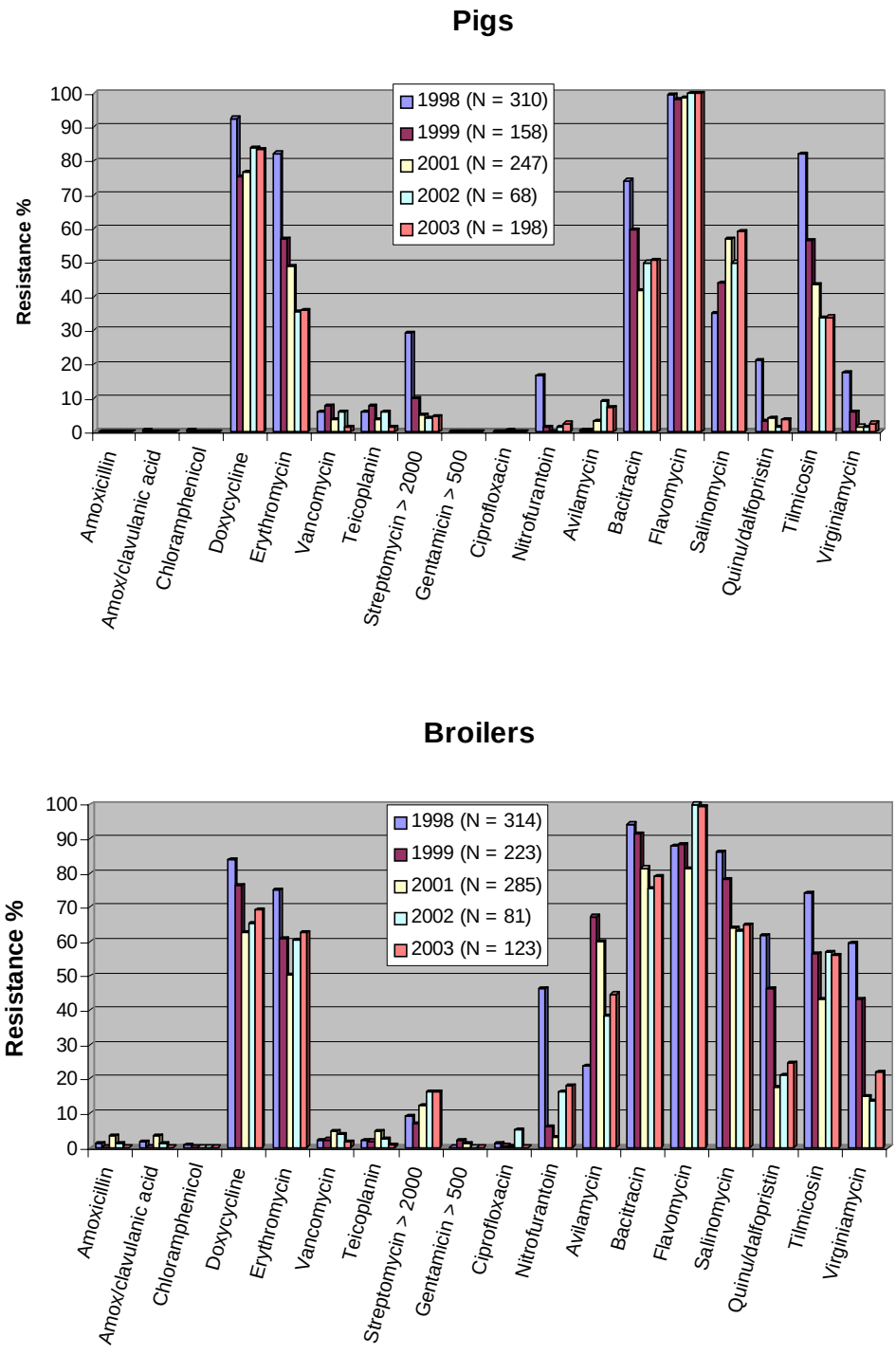


Figure 14. MIC distributions of salinomycin for *E. faecium* strains from 1998 – 2003, The arrow indicates the new breakpoint MIC (4 µg/ml).

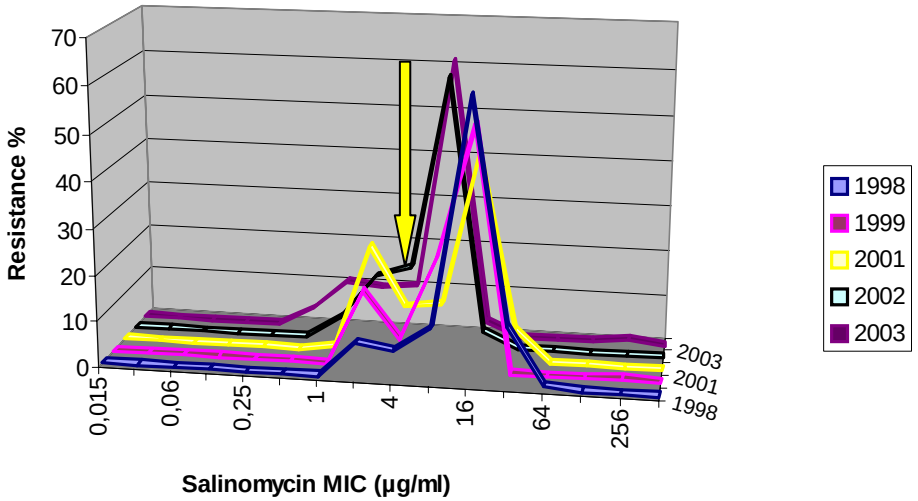
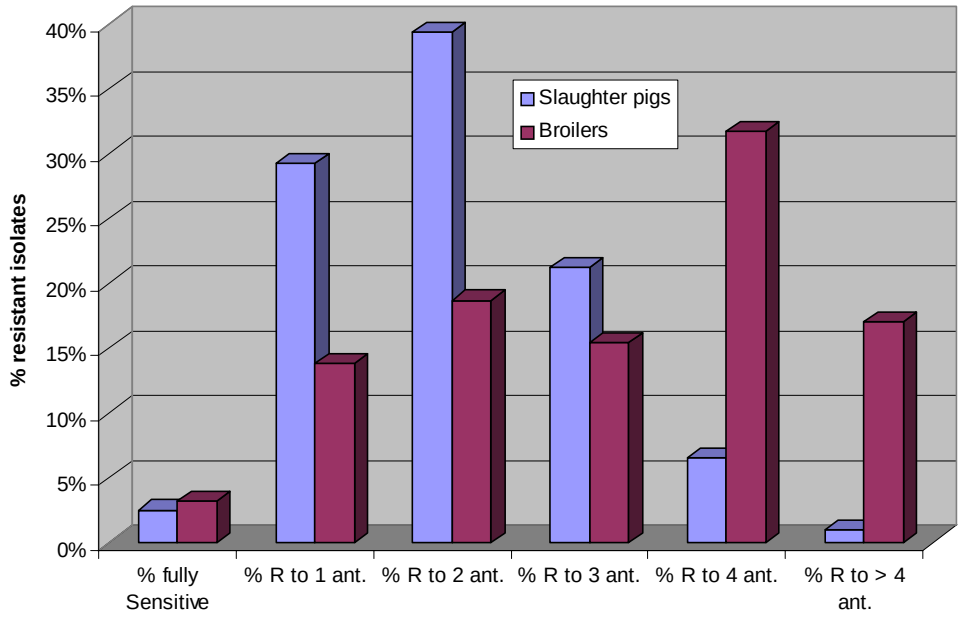


Figure 15. Percentages of *E. faecium* strains fully susceptible, resistant to one, two, three, four and more than four antibiotics in pigs and poultry in The Netherlands in 2003.



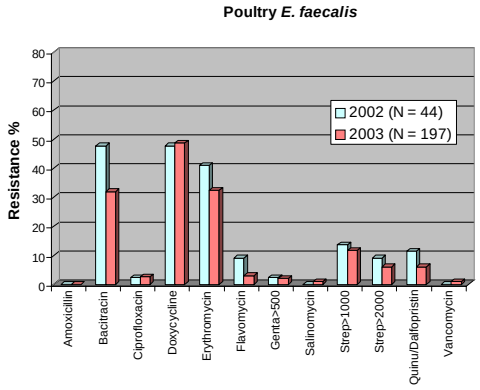
E. faecium and *E. faecalis* in raw meat products of food-animals

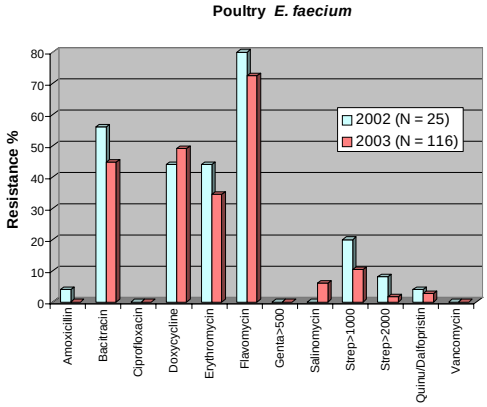
Table 15. Resistance % of *E. faecalis* and *E. faecium* isolated from raw meat products from poultry, beef and pork in the Netherlands in 2003

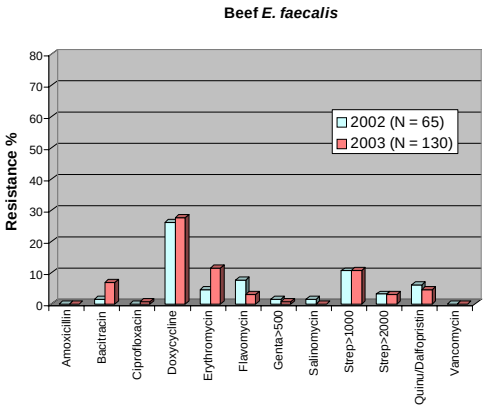
E. faecalis	**Poultry N = 197**	**Beef N = 130**	**Pork N = 54**
Amoxicillin	0	0	0
Doxycycline	48,7	27,7	35,2
Erythromycin	32,5	11,5	11,1
Vancomycin	1,0	0	0
Streptomycin > 1000 µg/ml	11,7	10,8	5,6
Streptomycin > 2000 µg/ml	6,1	3,1	1,9
Gentamicin > 500 µg/ml	2,0	0,8	0
Ciprofloxacin	2,5	0,1	0
Bacitracin	32,0	6,9	11,1
Flavomycin	3,0	3,1	13,0
Salinomycin	1,0	0	1,9
Quinu/dalfopristin	6,1	4,6	3,7
% fully susceptible	32,8	84,3	53,3
% R to 1 antibiotic	22,4	13,7	36,7
% R to 2 antibiotics	25	1	3,3
% R to 3 antibiotics	14,7	1	3,3
% R to 4 antibiotics	5,2	0	3,3
% R to > 4 antibiotics	0	0	0
E. faecium	**Chicken N = 116**	**Cattle N = 102**	**Pigs N = 30**
Amoxicillin	0	0	0
Doxycycline	49,1	4,9	23,3
Erythromycin	34,5	8,8	30,0
Vancomycin	0	0	0
Streptomycin > 1000 µg/ml	10,3	2,0	3,3
Streptomycin > 2000 µg/ml	1,7	1,0	3,3
Gentamicin > 500 µg/ml	0	0	0
Ciprofloxacin	0	0	0
Bacitracin	44,8	3,9	10,0
Flavomycin	72,4	92,2	93,3
Salinomycin	6,0	0	0
Quinu/dalfopristin	2,6	0	0
% fully susceptible	28,4	70	82,4
% R to 1 antibiotic	31,6	17,7	15,7
% R to 2 antibiotics	26	7,7	2
% R to 3 antibiotics	8,4	3,8	0
% R to 4 antibiotics	3,7	0,8	0
% R to > 4 antibiotics	1,9	0	0

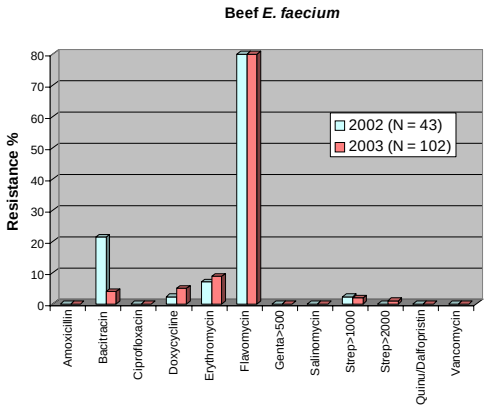
Resistance percentages in *E. faecium* isolated from raw meat products are lower than those found in isolates from food-animals. This may be selection bias due to the relatively small numbers tested. It may also indicate that subpopulations of strains adapted to survival in meat products exist. Vancomycin resistant strains were not found in meat products. Resistance percentages in isolates from cattle products were lower than those from the poultry and pig products. Resistance levels in *E. faecalis* were similar to those from *E. faecium* except for bacitracin and doxycycline from cattle. Resistance to flavomycin is lower in *E. faecalis*, because *E. faecium* is intrinsically resistant to this antibiotic.
Trend analysis is not possible because monitoring in raw meat products started in 2002.

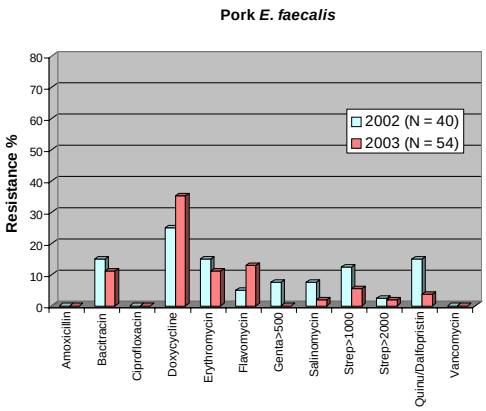
Figure 16. Trends in resistance percentages in *E. faecalis* and *E. faecium* isolated from raw meat products from poultry , beef and pork in The Netherlands from 2002 to 2003

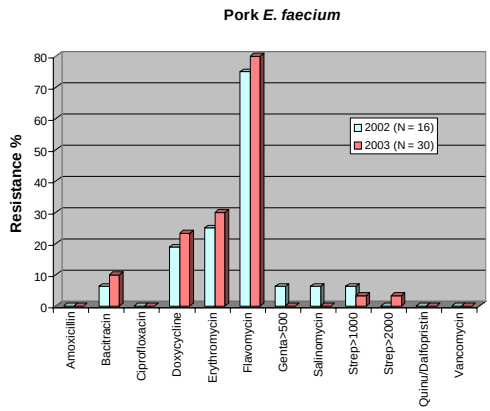












Animal pathogens

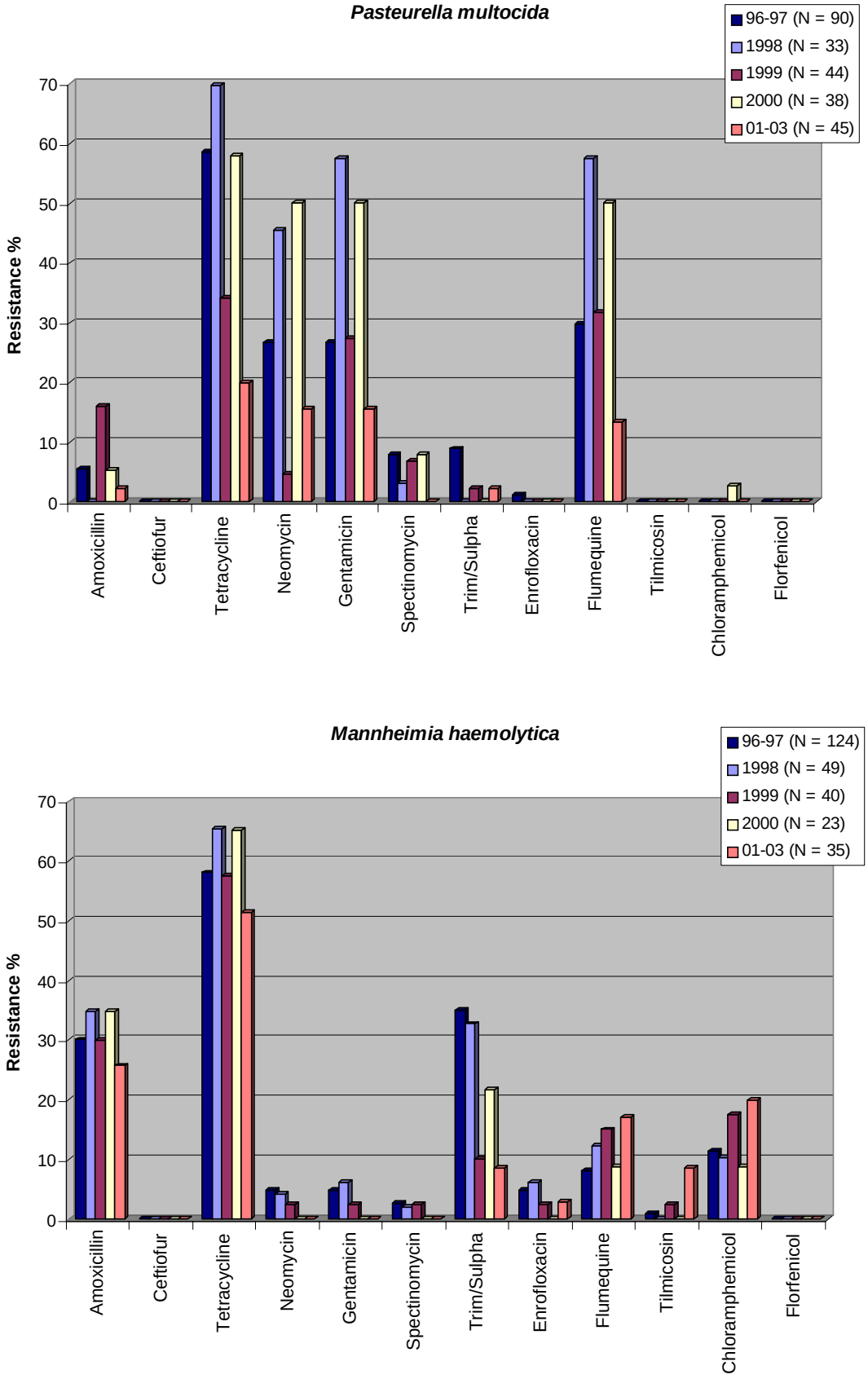
Respiratory tract pathogens*: Pasteurella multocida, Mannheimia haemolytica*

Table 16. MIC distribution (in %) for *Pasteurella multocida* (N = 45) and *Mannheimia haemolytica* (N = 35) isolated from clinically infected cattle in The Netherlands from 2001 - 2003

P. multocida 2001 - 2003	**MIC distribution (µg/ml)**															
	0,015	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Amoxicillin					93,3	2,2				2,2			2,2			2,2
Ceftiofur			84,4	13,3	2,2											0
Neomycin						2,2	11,1	22,2	35,6	13,3		2,2	11,1	2,2		15,6
Gentamicin						6,7	33,3	35,6	8,9	2,2			8,9	4,4		15,6
Spectinomycin									6,7	24,4	48,9	20,0				0
Tetracycline					2,2	37,8	13,3	15,6	6,7	4,4	8,9	6,7	4,4			20,0
Tilmicosin						11,1	26,7	24,4	22,2	15,6						0
Trim/sulpha				80,0	4,4		2,2	11,1		2,2						2,2
Flumequin					66,7	8,9	6,7	4,4					13,3			13,3
Enrofloxacin		77,8	8,9				13,3									0
Chloramphenicol					2,2	28,9	53,3			2,2	13,3					0
Florfenicol					6,7	93,3										0
M. haemolytica 2001 - 2003	**MIC distribution (µg/ml)**															
	0,015	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Amoxicillin					74,3								25,7			25,7
Ceftiofur			97,1	2,9												0
Neomycin								5,7	77,1	17,1						0
Gentamicin							40,0	60,0								0
Spectinomycin											88,6	11,4				0
Tetracycline					8,6	40,0					11,4	25,7	14,3			51,4
Tilmicosin						5,7		11,4	51,4	22,9		5,7		2,9		8,6
Trim/sulpha				60,0	14,3	5,7	11,4				5,7	2,9				8,6
Flumequin					62,9	8,6			11,4	8,6	2,9		5,7			17,1
Enrofloxacin		68,6	2,9		8,6	11,4	2,9		2,9	2,9						5,8
Chloramphenicol						5,7	40,0	34,3				17,1	2,9			20,0
Florfenicol						22,9	74,3	2,9								0

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

Figure 17. Trends in resistance percentages of *Pasteurella multocida* and *Mannheimia haemolytica* isolated from cattle in the Netherlands from 1996 - 2003



Since 1996, annually, clinical isolates of *Pasteurella multocida* (PMU) and *Mannheimia haemolytica* (MHA) strains isolated and identified by the Animal Health Service in Deventer, The Netherlands, are sent to CIDC-Lelystad for quantitative susceptibility testing. Because the numbers of strains tested were quite small and the strains were all isolated from clinically affected animals of which most of them were already treated intensively with antibiotics, the data have to be interpreted knowing that they represent a worst-case scenario, and not necessarily reflect the susceptibility patterns of pasteurella's and mannheimia's in Dutch cattle.
In table 16 the MIC-distributions and resistance percentages for the strains isolated from 2000 – 2003 are displayed. The resistance profiles seem to be species specific. Resistance to neomycin and gentamicin was only observed in PMU while resistance to amoxicillin, tetracycline, tilmicosin, enrofloxacin and chloramphenicol was higher in MHA. For the newer drugs ceftiofur (also representing cefquinome) and florfenicol no acquired resistances were detected in the Netherlands until 2003. For tilmicosin and enrofloxacin moderate resistance was detected in MHA only. Resistance to the both quinolones, flumequin and enrofloxacin, show a trimodal distribution in MHA. This indicates that although 5,8% of the MHA strains were highly resistant to enrofloxacin, another 22,9% showed reduced susceptibility to enrofloxacin. It is questionable that when treating these isolates with enrofloxacin, the therapeutic effect would be optimal.
Fig. 16 presents the trends in resistance percentages from 1996 to 2003. In both species real trends cannot be observed. Specifically in *P. multocida* a large year-to-year variation in resistance percentages exists. This may be due to selection, because the strains were mostly isolated from animals that died of pneumonia at autopsy. Resistance to tilmicosin and the quinolones seems to increase in MHA, but also this phenomenon could be due to selection bias.

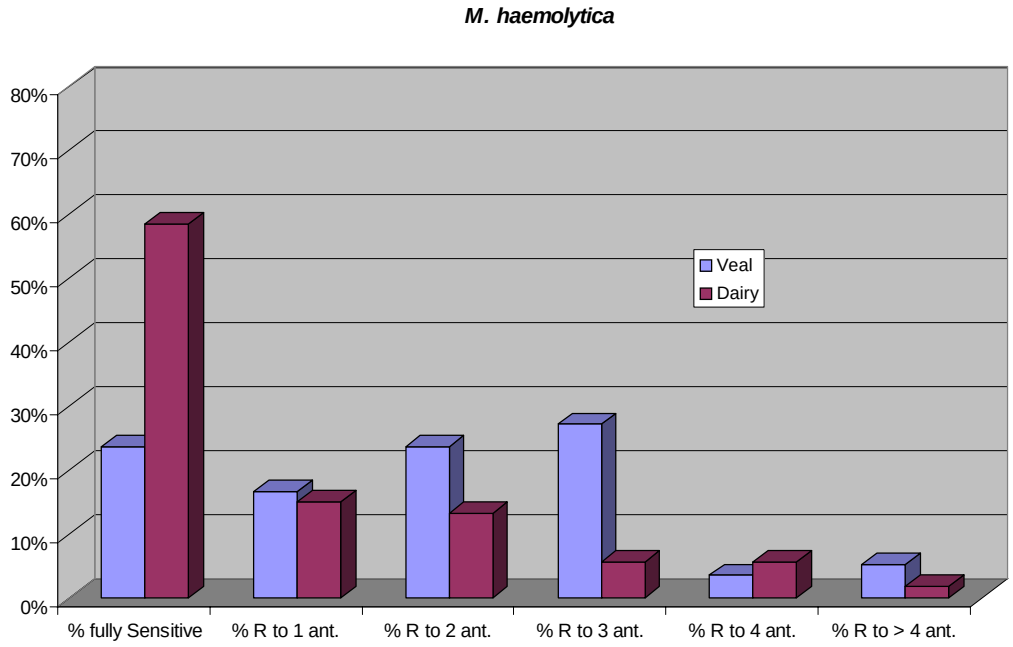
Table 17. Resistance % of *Mannheimia haemolytica* (MHA) and *Pasteurella multocida* (PMU) isolated from veal calves and cattle in the Netherlands from 1996 - 2003

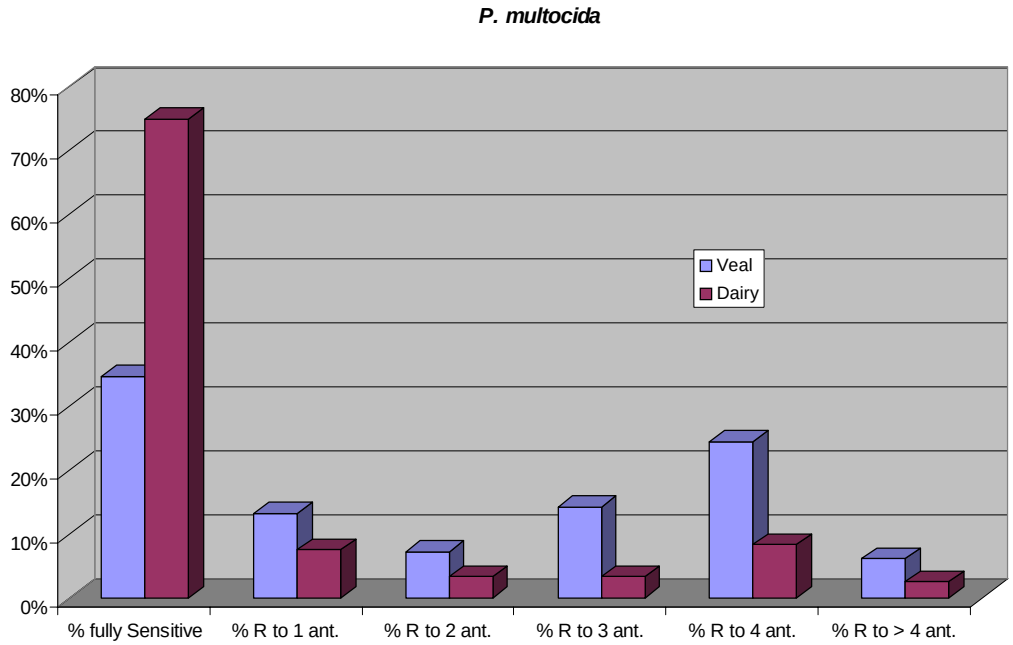
1996 – 2003	Veal calves		Other cattle	
	MHA	PMU	MHA	PMU
Number of strains	**114**	**98**	**107**	**121**
Amoxicillin	40	12,4	16,8	1,7
Ceftiofur	0	0	0	0
Tetracycline	74,8	48,5	37,4	18,3
Neomycin	4,4	35,7	1,9	14,2
Gentamicin	5,3	44,9	0,9	15,0
Spectinomycin	2,6	7,1	0	2,5
Trim/Sulpha	32,5	2	13,1	0
Enrofloxacin	6,1	1,0	2,8	0
Flumequin	14,0	48,0	9,3	15,7
Tilmicosin	0,9	0	3,7	0
Chloramphenicol	15,1	1,1	8,2	0
Florfenicol	0	0	0	0

In Table 17 a comparison is made between the resistance levels for pasteurella's and mannheimia's, isolated from veal calves, the bovine population with the highest consumption of antibiotics, and other cattle. In Fig. 17 the multi-resistance profiles of PMU and MHA from veal and other bovine sources are displayed. For both bacterial species the resistance percentage are higher in strains from veal calves reflecting the higher selection pressure in these animals, except resistance to tilmicosin, which is lower in strains from veal calves.

The percentage of strains resistant to three or more antibiotics were much higher in veal calves than in other bovine sources (fig 17). The percentage of multi-resistant MHA isolates was 36% for veal and 13,2% for other bovine sources. In PMU the levels were 44,9% and 14,2%, respectively.

Figure 18. Percentages of *M. haemolytica* and *P. multocida* strains fully susceptible, resistant to one, two, three, four and more than four antibiotics in veal and dairy calves in The Netherlands in 1996 - 2003.





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

Table 18. MIC-distributions (in %) for *E. coli* and coliform bacteria isolated from mastitis milk samples from Dutch cattle by the Animal Health Service in Deventer in 2003.

	MIC-distribution (µg/ml)															
E. coli N = 101	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Amoxicillin							3,0	33,7	34,7	7,9	2,0	1,0	17,8			18,8
Amox/clav. acid							3,0	44,6	36,6	12,9	2,0	1,0				1,0
Cefquinome			91,1	7,9		1,0										0
Cefoperazone			3,0	24,8	43,6	8,9	3,0	6,9	1,0	1,0	7,9					0
Cefuroxime						1,0	1,0	4,0	66,3	25,7	1,0	1,0				1,0
Tetracycline							23,8	31,7	23,8			1,0	19,8			20,8
Gentamicin					4,0	53,5	35,6	5,9		1,0						0
Kanamycin							2,0	15,8	54,5	16,8	4,0	6,9				6,9
Neomycin					1,0		51,5	35,6	4,0	1,0		6,9				6,9
Streptomycin									7,9	66,3	5,0	1,0	3,0	16,8		19,8
Enrofloxacin		60,4	35,6		1,0	1,0	2,0									0
Trim/sulpha				81,2	4,0	4,0						10,9				10,9
	MIC-distribution (µg/ml)															
Coliform N = 100	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Amoxicillin						4,0	3,0	5,0	5,0	1,0	5,0	11,0	66,0			77,0
Amox/clav. acid						5,0	20,0	38,0	7,0	1,0	3,0	7,0	18,0	1,0		26,0
Cefquinome			85,0	9,0	3,0		2,0	1,0								0
Cefoperazone			8,0	12,0	37,0	15,0	7,0	9,0	6,0	2,0	4,0					0
Cefuroxime						1,0	14,0	31,0	19,0	13,0	5,0	17,0				17.0
Tetracycline					1,0	10,0	35,0	30,0	7,0	5,0		6,0	6,0			12,0
Gentamicin				7,0	69,0	21,0	3,0									0
Kanamycin				1,0		4,0	40,0	42,0	7,0	3,0		3,0				3,0
Neomycin				1,0	4,0	60,0	30,0	3,0	1,0			1,0				1,0
Streptomycin						1,0	2,0	17,0	60,0	7,0	3,0	2,0	4,0	4,0		8,0
Enrofloxacin		54,0	34,0	7,0	3,0	2,0										0
Trim/sulpha				87,0	6,0	5,0	1,0					1,0				1,0

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

E. coli isolated from milk samples from cows suffering from mastitis were in general susceptible to the antibiotics included in the panel. Only resistance to amoxicillin, streptomycin, trim/sulpha and tetracycline was present in significant percentages. All strains were susceptible to the 3rd generation cefalosporins (cefquinome and cefoperazone) tested, although the cefoperazone MICs show a subpopulation of 7,9% potentially resistant. All isolates were susceptible to enrofloxacin and gentamicin. The coliform bacteria (27 *Enterobacter* , 63 *Klebsiella,* 10 other species) showed a high level of resistance to amoxicillin (all klebsiella's are ß-lactamase producers), and to the combination with clavulanic acid (predominantly *Enterobacter* and other species). Regarding the other antibiotics tested, only the levels of resistance to cefuroxime and tetracycline were noteworthy. Fig. 18 shows that no trend in resistance from 2002 to 2003 could be observed.

Figure 19. Trends in resistance percentages for *E. coli* and coliform bacteria isolated from mastitis milk in the Netherlands from 2002 – 2003.

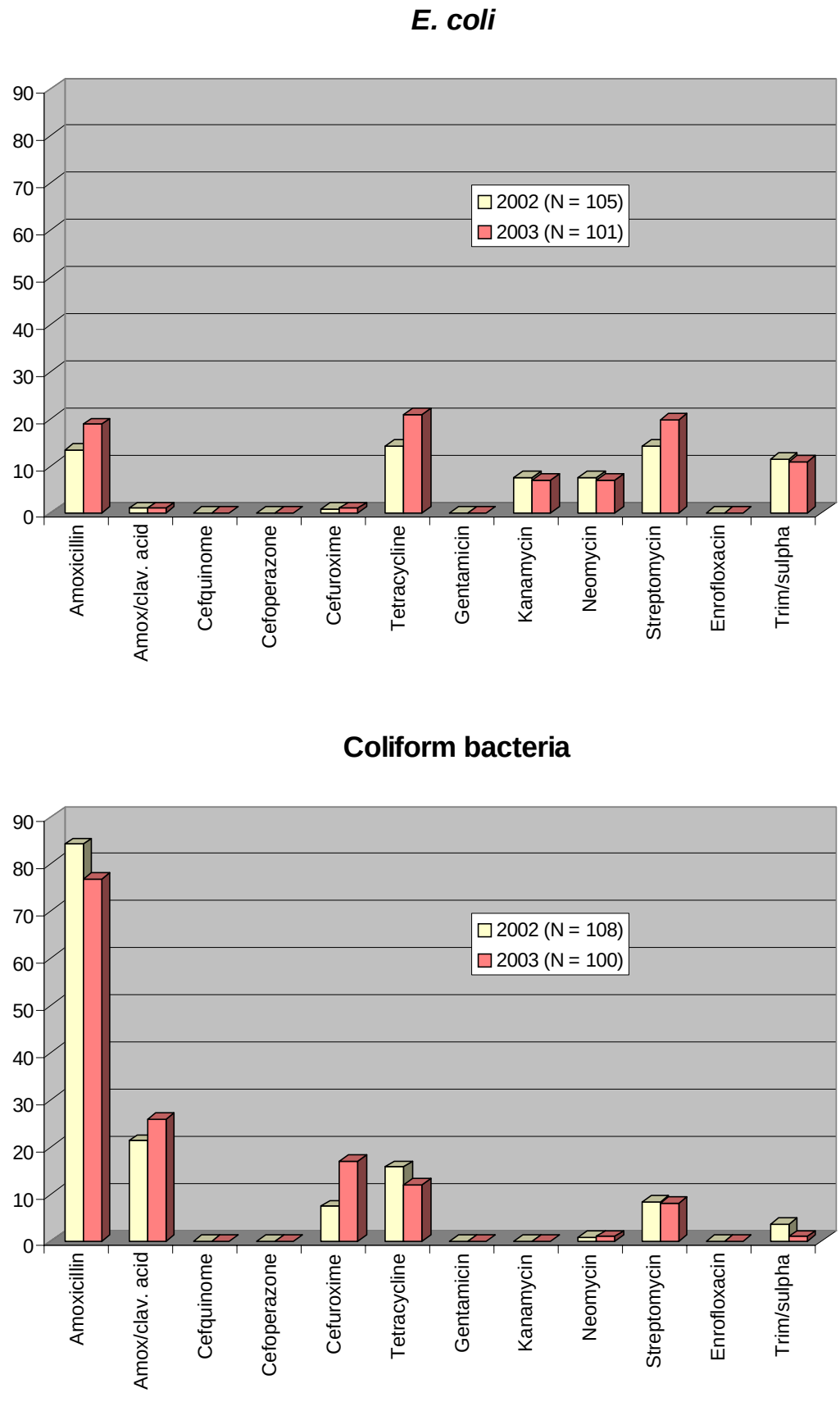


Table 19. MIC-distributions (in %) of *S. aureus* and coagulase-negative staphylococci isolated from mastitis milk samples from Dutch cattle by the Animal Health Service in Deventer in 2003.

	MIC-distribution (µg/ml)															
***S. aureus* (N = 107)**	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R**
Penicillin	-	-	74,8	0,9	1,9	-	-	-	-	7,5	15,0	-	-	-	-	24,3
Oxacillin	-	-	-	14,0	43,0	36,4	5,6	0,9	-	-	-	-	-	-	-	0
Amox/clav. Acid	-	-	-	49,5	26,2	1,9	17,8	4,7	-	-	-	-	-	-	-	0
Cephalothin	-	-	-	26,2	43,9	27,1	2,8	-	-	-	-	-	-	-	-	0
Tetracycline	-	-	-	0,9	0,9	85,0	9,3	0,9	-	-	0,9	1,9	-	-	-	2,8
Kanamycin	-	-	-	-	-	-	2,8	50,5	44,9	1,9	-	-	-	-	-	0
Neomycin	-	-	-	0,9	27,1	54,2	15,9	1,9	-	-	-	-	-	-	-	0
Streptomycin	-	-	-	-	-	-	-	-	9,3	62,6	26,2	-	1,9	-	-	1,9
Erythromycin	-	-	-	0,9	3,7	90,7	4,7	-	-	-	-	-	-	-	-	0
Lincomycin	-	-	-	-	-	2,8	88,8	2,8	-	-	0,9	4,7	-	-	-	5,6
Pirlimycin	-	-	-	-	11,2	77,6	7,5	0,9	2,8	-	-	-	-	-	-	2,8
Trim/sulpha	-	-	-	94,4	3,7	1,9	-	-	-	-	-	-	-	-	-	0
	MIC-distribution (µg/ml)															
***Coag. neg. staph.* (N = 92)**	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R**
Penicillin	-	-	68,5	3,3	7,6	-	3,3	5,4	6,5	2,2	3,3	-	-	-	-	28,3
Oxacillin	-	-	3,3	22,8	48,9	18,5	4,3	-	1,1	-	1,1	-	-	-	-	25,0
Amox/clav. Acid	-	-	-	66,3	25,0	6,5	1,1	1,1	-	-	-	-	-	-	-	0
Cephalothin	-	-	4,3	42,4	34,8	13,0	5,4	-	-	-	-	-	-	-	-	0
Tetracycline	-	-	-	4,3	22,8	56,5	2,2	2,2	1,1	-	1,1	9,8	-	-	-	10,9
Kanamycin	-	-	-	6,5	14,1	28,3	27,2	19,6	2,2	-	1,1	1,1	-	-	-	1,1
Neomycin	-	-	-	68,5	27,2	3,3	-	-	-	1,1	-	-	-	-	-	0
Streptomycin	-	-	-	-	0,0	3,3	15,2	28,3	29,3	15,2	3,3	2,2	3,3	-	-	5,4
Erythromycin	-	-	-	2,2	29,3	56,5	4,3	1,1	-	4,3	-	2,2	-	-	-	6,5
Lincomycin	-	-	-	-	2,2	23,9	38,0	10,9	6,5	3,3	5,4	9,8	-	-	-	18,5
Pirlimycin	-	-	-	7,6	62,0	14,1	5,4	5,4	2,2	2,2	-	1,1	-	-	-	5,4
Trim/sulpha	-	-	-	67,4	21,7	7,6	-	-	1,1	-	-	2,2	-	-	-	3,3

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. The vertical bars indicate the breakpoints.

In spite of the intensive use of antibiotics in the control of bovine mastitis in The Netherlands, the *S. aureus* isolates tested were susceptible to most antibiotics. 24,3% of the isolates were penicillinase producers but oxacilllin resistance was not present. 5,6% were resistant to lincomycin and 2,8% to the related but more potent lincosamide drug, pirlimycin. The coagulase negative staphylococci (24 *S.* chromogenes, 16 *S. epidermidis,* 15, *S. hyicus,* 13 *S. xylosis,* 34 other species) were more resistant than *S. aureus.* 28,3% were resistant to penicillin and 25% to oxacillin. The relatively high prevalence of oxacillin resistance will partly be due to the presence of the *MecA*-gene and partly by intrinsically lower susceptibility and the lower breakpoint for coagulase negative staphylococci . The MIC-distribution for oxacillin demonstrates that only 2 isolates were highly resistant.
Resistance to lincomycin was quite common (18,5%), resistance to pirlimycin was substantially lower (5,4%).

Although the numbers of strains included were relative large, the trends in resistance in fig. 20 may be due to selection bias and not reflect true trends.

Figure 20. Trends in resistance percentages for *S. aureus* and coagulase negative staphylococci isolated from mastitis milk in The Netherlands in 2002 and 2003

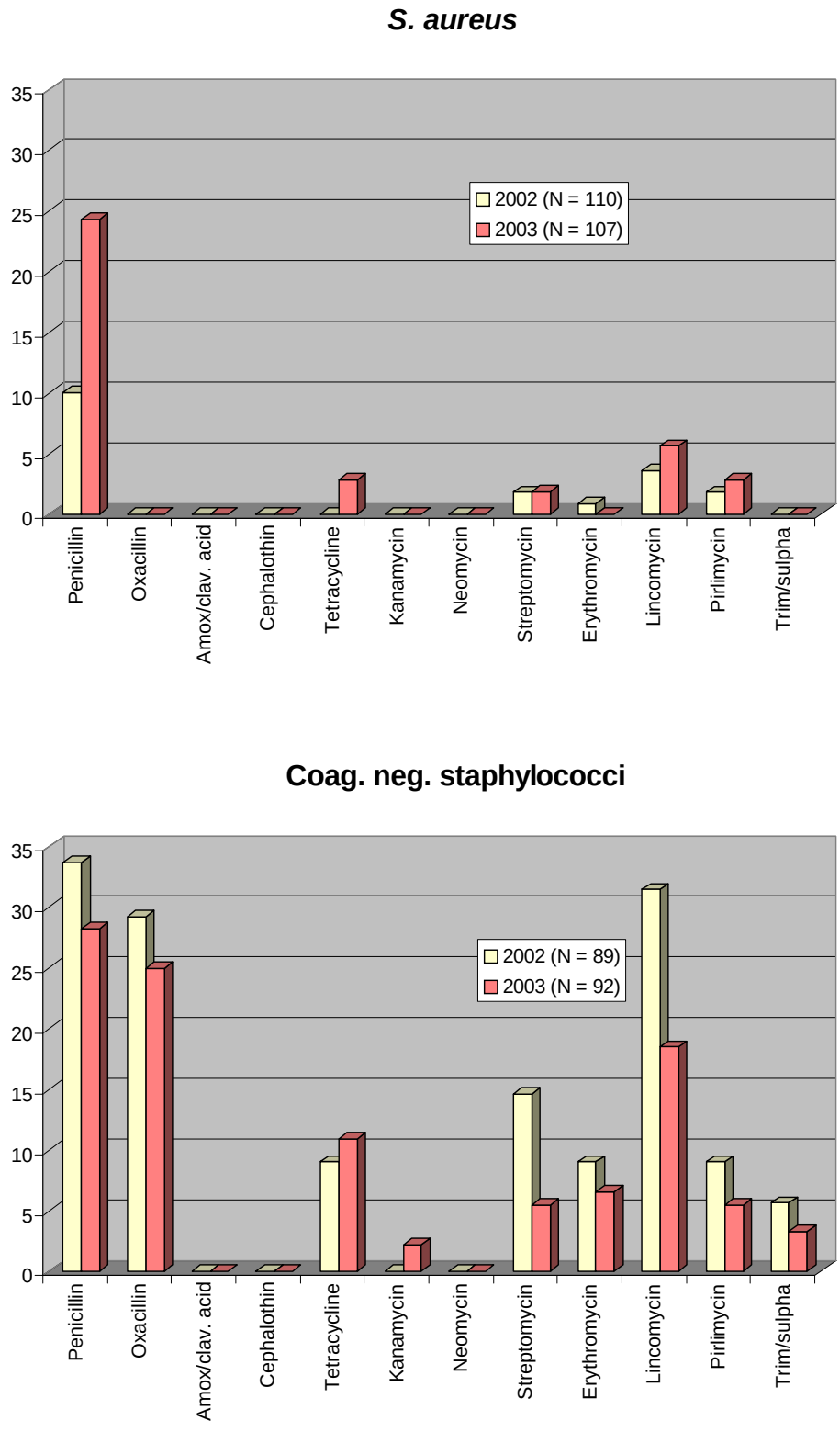


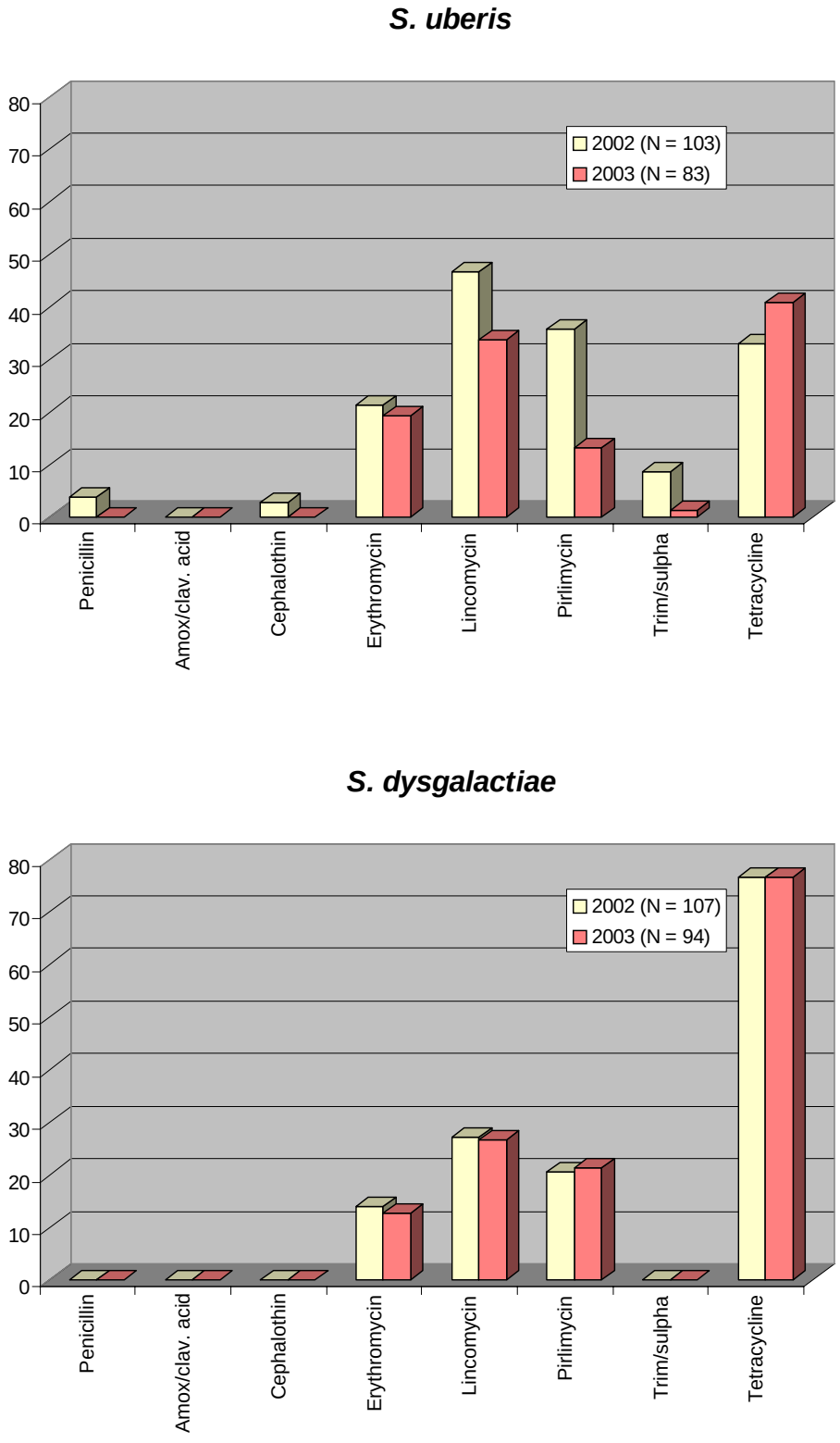
Table 20. MIC-distributions (in %) of *S. uberis* and *S. dysgalactiae* isolated from mastitis milk samples from Dutch cattle by the Animal Health Service in 2003.

	MIC-distribution (µg/ml)															
S. uberis (N = 83)	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Penicillin	66,3	3,6	12,0	10,8	6,0	1,2	.	.	.	.	.	.	.	.	.	0
Amox/clav. acid	43,4	24,1	4,8	20,5	6,0	.	.	.	.	.	.	.	.	.	.	0
Cephalothin	6.0	8,4	16,9	24,1	9,6	12,0	22,9	.	.	.	.	.	.	.	.	0
Erythromycin	6,0	20,5	47,0	7,2	.	.	3,6	2,4	2,4	.	.	1,2	9,6	.	.	19,3
Lincomycin	1,2	3,6	10,8	14,5	3,6	2,4	4,8	19,3	6,0	4,8	2,4	.	26,5	.	.	38,7
Pirlimycin	10,8	9,6	34,9	9,6	3,6	3,6	2,4	12,0	7,2	2,4	.	.	3,6	.	.	13,3
Trim/sulpha	.	1,2	13,3	39,8	34,9	9,6	.	.	1,2	.	.	.	.	.	.	1,2
Tetracycline	.	.	1,2	12,0	26,5	19,3	.	.	.	1,2	3,6	20,5	12,0	3,6	.	41,0
	MIC-distribution (µg/ml)															
S. dysgalactiae (N = 94)	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Penicillin	97,9	.	.	1,1	1,1	.	.	.	.	.	.	.	.	.	.	0
Amox/clav. acid	96,8	1,1	.	1,1	1,1	.	.	.	.	.	.	.	.	.	.	0
Cephalothin	1,1	1,1	7,5	86,0	3,2	.	2,2	.	.	.	.	.	.	.	.	0
Erythromycin	.	21,3	57,4	7,4	.	1,1	.	.	.	.	.	.	12,9	.	.	12,8
Lincomycin	.	.	1,1	24,5	33,0	1,1	1,1	1,1	11,7	4,3	1,1	.	21,3	.	.	26,6
Pirlimycin	.	4,3	50,0	20,2	4,3	.	.	.	2,1	5,3	1,1	.	12,8	.	.	21,3
Trim/sulpha	.	1,1	1,1	38,3	47,9	11,7	.	.	.	.	.	.	.	.	.	0
Tetracycline	.	.	.	.	.	1,1	2,1	7,4	12,8	3,2	1,1	16,0	56,4	.	.	76,6

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

In 2003 94 *S. uberis* strains were sent to CIDC to determine the resistance profiles. After conformation of the identification, 4 of these isolates were enterococci, 3 isolates were lactococci and 1 isolate was a *S. dysgalactiae*. Of the remaining 83 isolates, two were identified with PCR as *S. parauberis*. The 84 strains were more frequently resistant to erythromycin, lincomycin, pirlimycin and trim/sulpha than *S. dysgalactiae*. Resistance to tetracycline was highest in *S. dysgalactiae*. It is quite essential to differentiate adequately between *S. uberis* and enterococci and lactococci because the latter two species are intrinsically more resistant to antibiotics. The inclusion of these species may overestimate resistance being present in populations examined.
The observed differences in resistance percentages of the lincosamides and trimethoprim-sulphamethoxazole for *S. uberis* between 2002 and 2003 are striking (fig. 20), but again it may be part of the normal variation and not represent a real trend.

Figure 21. Trends in resistance percentages for *S. uberis* and *S. dysgalactiae* isolated from mastitis milk in The Netherlands in 2002 and 2003



III Discussion and Conclusion

Veterinary antimicrobial resistance as a public health threat
Development of antimicrobial resistance is considered to be one of the major public health threats. It is a consequence of selective pressure on bacteria by exposure to antimicrobial agents. By genetic exchange mechanisms resistance can spread between bacteria. This spread is not inhibited by phylogenetic, ecological or geographical boundaries. Therefore development of resistance in the animal reservoir may have an impact on the resistance development in bacteria regularly infecting humans and may ultimately interfere with the treatment of infectious diseases in humans. This can happen directly as zoonotic agents become resistant, or indirectly when commensal animal bacteria transfer their resistance to human bacteria. For this reason a continuous monitoring of the development of resistance in bacteria of the veterinary and agricultural sector that may have an impact on public health is necessary. The exposure of animals to antibiotics is one of the main factors contributing to resistance. For policy making it is important to identify this hazard, therefore a system to obtain detailed data on the exposure of animals to antibiotics is also necessary.

Usage of antibiotics
In 2003, for the first time since 1999 when antibiotic usage in livestock started to be measured in The Netherlands, the total amount of therapeutic use of veterinary antibiotics has decreased by 2% since 2002. However, in 2003 the numbers of kg's of food animals produced in The Netherlands have decreased as well. The main antibiotic classes used in food producing animals were as before tetracyclines and trimethoprim-sulphonamide combinations. Quinolone usage was 5000 kg in 2003, 272 kg of which were fluoroquinolones used in poultry. This seems only a very small proportion of the total amount of antibiotics used. However, with a dosage for fluoroquinolones of 10 mg/kg body weight, with 272 kg of fluoroquinolones, 27 million broilers and other poultry of 1 kg bodyweight were exposed once. Moreover a similar amount of animals were exposed to flumequin. Therefore the total exposure of poultry to quinolones in 2003 was substantial.
The use of antimicrobial growth promoters decreased from 250.000 kg in 1998 to 75.000 kg in 2003.

Both Denmark and The Netherlands have restrictive antibiotic policies and are considered to be countries where antibiotic usage in livestock is low. Denmark produces very accurate usage statistics since 1997. Comparing usage data from The Netherlands with data from Denmark shows that the therapeutic use of antibiotics in pig, cattle and broiler production is more intensive in The Netherlands. This is not necessarily a judgment. Administration of antibiotics has risks and benefits. A risk is development of resistance of public health concern; a benefit of using antibiotics is increased animal welfare. These factors are difficult to weigh without more quantitative knowledge on risks and benefits.
The reason why in Denmark fewer antibiotics are used for therapeutic purposes is subject for debate. In The Netherlands the usage of antibiotics in veal calves, an industry that is lacking in Denmark, is considered to be a major factor in explaining the observed difference. The way Dutch veal calf industry is organized is different from the more extensive way beef is produced in Denmark and results in more intensive use of antibiotics. The observed difference may also be explained by the different distribution system of antibiotics in Denmark, the lower incidence of diseases or the fact that virtually every administration of antibiotics is monitored in Denmark, which may have a positive effect on prescription by veterinarians..

Trends of resistance in the Netherlands
Data from 2003 in the current report indicate a slow overall increase in quinolone (**nalidixic acid/flumequin)** resistance (all nalidixic acid/flumequin-resistant strains demonstrate decreased susceptibility to ciprofloxacin) in salmonella's in The Netherlands. This increase is serotype specific. In *S.* Typhimurium quinolone resistance was limited to isolates from human infections and poultry products. In *S.* Java, the very rapid increase in quinolone resistance from 0% in 2000 to 35% in 2002, stabilised in 2003 (34,2%) in strains from broilers. None of these isolates demonstrated high level ciprofloxacin resistance as was observed in Germany. In *S.* Enteritidis a major increase in quinolone-resistance was observed, predominantly in human isolates of Pt1, 6a, 8, and 14b related to imported products and travel. In Dutch layers no quinolone resistant *S.* Enteritidis strains were detected.
Ciprofloxacin-resistance in *C. jejuni* strains from broilers in 2003 slightly increased to 41,7%. The ciprofloxacin resistance percentages in human clinical isolates slowly increased in the 1990s to app. 25%, from 2000 until 2003 the level was stable around 31%. For acquisition of human infections with fluoroquinolone-resistant campylobacters, travel contributed to a higher proportion than domestic acquisition (MARAN 2002).
Quinolone resistance in food borne commensal organisms was mainly observed in *E. coli* from broilers and broiler products. In this animal species approximately 35% of all *E. coli*'s demonstrated reduced susceptibility to ciprofloxacin and 3% high level resistance to this drug. In Pigs no quinolone resistance was observed in *E. coli.* This reflects the selection pressure through usage of enrofloxacin and flumequin in broilers.

Resistance to **cefotaxime** (ESBL-positive) was found, both in human and poultry salmonella's (N = 13). These isolates belonged to the following serotypes: 4 *S.* Virchow, 2 *S.* Paratyphi B var. Java and 1 *S.* Bareilly from poultry, 2 *S.* Typhimurium ft 507 and 508, respectively (Dutch phage typing system), 2 *S.* Concord and 1 *S.* Enteritidis pt4 isolated from human patients and 1 *S.* Enteritidis pt21 from an unknown source. The majority of these isolates were multiple resistant to amoxicillin, cefotaxime (ESBL-positive), tetracycline, trimethoprim, sulphamethoxazole and chloramphenicol. Six of these isolates were also resistant to nalidixic acid, including all *S.* Virchow's, one *S.* Java and *S.* Enteritidis pt21.
It is the second consecutive year that ESBL-positive *S.* Java strains were detected. Because it is a clone spreading in broilers in Germany and The Netherlands only, it indicates that these isolates were selected locally by transfer of plasmid mediated ESBLs from other *Enterobacteriaceae* and not imported.
In randomly picked *E. coli* strains from broilers cefotaxime-resistance was present less frequently than in 2002 (2,4% versus 6,1% in 2002). In broilers four cefotaxime resistant strains were isolated that were suspected to be ESBL-positive, and in pigs one.

Resistance to **vancomycin** in *E. faecium* was low and showed a tendency to further decrease. However vancomycin resistance at these low levels will most probably be present on most of the broiler and pig farms in The Netherlands and may easily be selected again after reintroduction of selective agents. Resistance to the other growth promoters stabilized after the initial decrease related to the ban in 1999. Since 1999, after the ban of macrolides, virginiamycin and bacitracin, avilamycin was likely to be used more frequently in broilers resulting in a rapid increase in resistance in *E. faecium* from 22% in 1998 to 67% in 1999. After 1999 the avilamycin resistance in broilers slowly decreased again to 60% in 2001 and 38% in 2002, followed by an increase to 44,7% in 2003

Multiple-resistance. In MARAN-2003 for each species involved an analysis of the percentages of multiple resistances was performed. In the foodborne commensal organisms of the animal species included the percentages of multiple resistant strains were much higher in broilers than in pigs. This reflects both the intensive use of antibiotics in broilers but also the husbandry system. In broilers after any selection pressure by treatment with antibiotics, time is lacking to restore a more susceptible gut flora, because the animals are slaughtered after about 6 weeks. Multiple resistance was more commonly present in *P. multocida* and *M. haemolityca* from veal calves than from dairy cattle, again reflecting the different use practices in these animals.

Comparing the resistance data from this report with DANMAP 2003 demonstrates that effects of the ban of the growth promoters in 1999 are quite similar. After an initial relative rapid decrease, the resistance levels in *E. faecium* seem to stabilise. In general in Dutch strains of foodborne pathogens and commensals the resistance levels are higher than in Danish strains. It may reflect the higher consumption pattern in The Netherlands.

As in DANMAP 2003, travel associated resistance also plays an important role in our data. In addition, the import of resistant strains on meat products is documented in DANMAP 2003. It stresses the necessity for The Netherlands to focus further on imported products in an attempt to quantify the contribution of the imported products to the resistance situation in The Netherlands.

Conclusions and recommendations

It can be concluded that the level of usage of antibiotics in food animals in The Netherlands is higher than in a country with a similar but less intensive husbandry system. In Dutch bacteria from food animals the resistance levels are higher.
Data from the present report indicate that the major risk factors for development of antimicrobial resistance of public health concern are antibiotic usage in poultry (both in The Netherlands and abroad), consumption of imported products and travel.

Based on the results of this report it can be recommended that:

- Usage and resistance data from veal calves need to be included in future reports;
- Resistance trends of quinolones in Salmonella, Campylobacter and *E. coli* in food animals and in humans warrant optimisation of prudent use policies;
- Imported food products should be included in the monitoring programme.
- More detailed data on therapeutic use of antibiotics in the different food animal species (pigs, poultry and veal calves) are needed to determine the real exposure to antibiotics.

IV Appendices

Appendix I. Tracing back human *Salmonella* Enteritidis infections to imported eggs, travel and country of origin using phagetyping and testing for nalidixic-acid resistance.

Quite a few publications in 2004 show the strong decrease since 1998 of the once predominant and highly successful phagetype 4 of *S.* Enteritidis (SE) in most European countries, to be replaced by a range of emerging new SE-phagetypes. Most noteworthy among the emerging SE-phagetypes are Pt-1, Pt-6, Pt-8, Pt-14b and Pt-21. It appears from data of travel-related infections, knowledge from some countries of locally circulating phagetypes in poultry and extensive testing of imported eggs (the main vehicle for transmission of SE) in the UK, that some hotspot-regions of origin of the emerging phagetypes can be indicated in Europe, especially when the phagetype demonstrates nalidixic-acid (Na) resistance.
In Holland surveillance programs show that the Salmonella control program in poultry has been successful in reducing SE in broilers almost to exclusion. However, in commercial layers in 2003 still more than 6% (9% in 2001 and 14% in 1997) of the flocks are SE-positive. Table 1 shows that most of the above-mentioned emerging phagetypes are now found in Dutch layer flocks but none have been tested positive for Na-resistance as yet. Other endemic sources for SE besides poultry hardly count in The Netherlands.
In humans however, Na-resistance is often found in SE-infections, most noteworthy related to Pt-1, Pt-6, Pt-6a and Pt-8. Hence, these cases of infection presumably can only be caused by imported foods and travel.

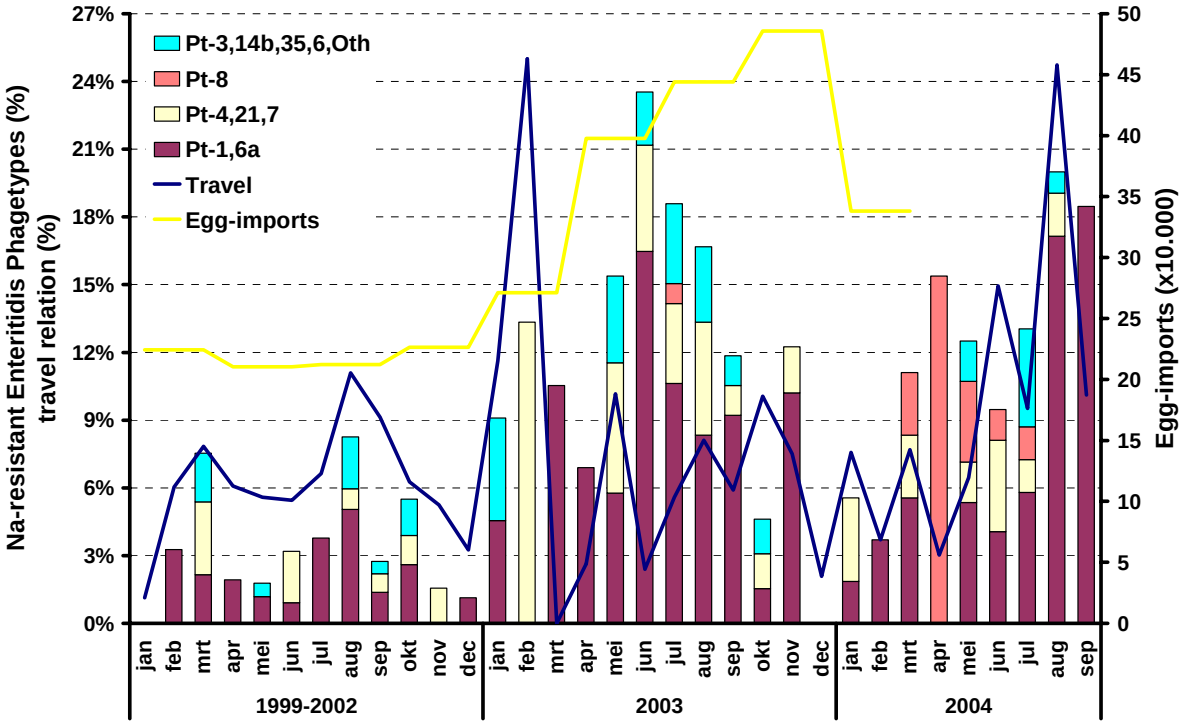
Table. 1 Nalidixic acid resistance in *S.* Enteritidis phagetypes in The Netherlands in humans and among Dutch broiler and layer flocks between 1999 and September 2004.

		Sometimes (F)Q-resistant Enteritidis phagetypes (Pt)													Never (F)Q-resist Pt's
		Enteritidis	Pt 35	Pt 1	Pt 7	Pt 6b	Pt 6a	Pt 3	Pt 8	Pt 6	Pt 14b	Pt 4	Pt 21	Other Pt's	
Antibiotic tests 1999 - 9/2004	Humans	2497	13	301	13	9	75	17	247	164	79	907	439	26	207
	Broilers	44	1	1	-	-	-	-	-	1	-	32	4	3	2
	Layers	140	3	4	11	-	-	-	3	7	6	77	2	3	24
Nalidixic acid resistance (%)	Humans	11%	54%	48%	46%	33%	20%	18%	8%	7%	5%	4%	3%	31%	0%
	Broilers	0%	-	-	-	-	-	-	-	-	-	-	-	-	-
	Layers	0%	-	-	-	-	-	-	-	-	-	-	-	-	-

In June 2003, a significant excess isolation rate of *Salmonella* Enteritidis (SE) was reported as compared to former years in most regional public health laboratories. At the end of 2003, this amounted to an extra 540 laboratory confirmed cases for the whole of the Netherlands, which would mean an estimated 7500 extra cases of gastro-enteritis caused by SE in the general population, which is an increase of 50%. It was estimated that the 2003 excess *S.* Enteritidis infections caused an extra 8-11 deaths.
Strong evidence is found that the increase in importation of contaminated eggs due to the avian influenza outbreak in poultry is the most probable reason for this excess. Figure 1 illustrates the reasoning. In the four years before 2003 monthly Na-resistance of SE isolates nicely followed the percentage of travel related cases. During the avian influenza outbreak (March-Mai 2003) imports of eggs strongly increased, Na-resistance increased to almost 24% predominantly due to Pt-1 and Pt-6a and clearly was not explained by travel. Imports of

Spanish eggs that are notorious for Pt-1 and PT-6a Na-resistant, strongly increased in the 2nd and 3rd quarter and decreased in the 4th quarter of 2003. Hence eggs imported from Spain are strongly implicated. Egg-imports were still high in the 1st quarter of 2004, especially from central European countries notorious for Na-resistant Pt-8. An excess of Pt-8 cases was seen in March to Mai 2004 in humans and indeed an increased Na-resistance due to Pt-8 is clearly implied in that period. In August-September 2004 again an excess of Pt-1 and PT-6a cases was observed accompanied with high levels of Na-resistance due to Pt-1 and Pt-6a. Travel now seems to be an important reason as well but can only partly explain the excess of cases. Data of imported eggs were not known at that time.

Figure 1. Monthly nalidixic-acid resistance and travel-relation of *S*. Enteritidis strains of infected humans, received at the Dutch National Salmonella Centre. Quarterly egg-imports in the four years



Salmonella is found in only about 0,03% of eggs produced in Holland and is still decreasing as SE contamination in layers has more than halved since 1997. Nevertheless 35% of human salmonellosis can be attributed to Dutch eggs. The reason that relatively small imports can have such a huge effect on Dutch human salmonellosis is that the level of contamination of Dutch eggs is 10 respectively 160 times lower than those in the UK and Spain.

In conclusion the above stresses that it is essential in decision making to be able to trace back the source of outbreaks of salmonellosis by testing for antimicrobial resistance, next to sero- and phagetyping of positive findings. Furthermore that testing should happen on a continuing basis both in the Dutch food chain as well as in imported foods.

Appendix II. Comparison of Usage of Antibiotics in The Netherlands and Denmark

Introduction

To benchmark antibiotic use in The Netherlands, we used Denmark as the reference country. Both Denmark and The Netherlands have restrictive antibiotic policies and are considered to be countries where antibiotic usage in livestock is low. Intensity of animal production and the climate in Demark is comparable to the Dutch situation. Last but not least, detailed Danish usage data are published yearly. In MARAN 2002 Danish consumption data over 2002 were compared with Dutch data over 2002. The mass of active substance used related to the live weight slaughtered, indicated that overall consumption of antibiotics in the Netherlands in food-animals was substantially higher than in Denmark. A more detailed comparison over 2002 is described here.

Data sources used

Danish data are published yearly in the DANMAP reports, which are considered to be a reliable source for Danish consumption data. These usage statistics are extracted from the VetStat system. In this system detailed consumption data supplied by veterinarians, pharmacists and feed mills are collected. In general the target species is known, allowing the expression of usage statistics as the number of animal daily dosages per species. In 2002 the quality of usage data of antibiotics sold or used by veterinary practices in particular was not validated. Therefore in DANMAP 2002 16,4% (15.166 kg) of the total antibiotic mass used in Denmark is reported ‘for use in veterinary practice, species not given’. This affects primarily the validity of cattle usage statistics since a large part of drugs used in cows are sold or administered by veterinarians. The majority of drugs used in poultry (66%) and calves (80%) and nearly all drugs in pigs are sold directly via pharmacies and feed mills. On the other hand, since only few veterinary practices are responsible for treating poultry, data from these practices are considered to be reliable (Jensen et al, in press).
As described earlier Dutch statistics on total sales of antibiotics for veterinary use are based on reports from pharmaceutical companies, represented by the FIDIN. These data are reliable, but do not include information on usage in individual target animal species.
Over 1999 more detailed data are available. Over this period a study using records from 24 Dutch veterinary practices was carried out; some results of this study were published in MARAN 2002. The sample included 3,3 % of the total Dutch pig population, 1,3 % of the poultry population and 17% of the Dutch cattle population. The sample size was relatively small and the quality of the population examined was not validated. Usage data on veal calves and aquaculture could not be obtained.
Livestock population statistics from Denmark are obtained at www.statbank.dk, data from the Netherlands at statline.cbs.nl. Danish animal production data are published at www.meatboard.dk. Dutch production data are available at bedrijfsnet.pve.agro.nl.

All animals

In The Netherlands in 2002, 402.000 kg antibiotics were used. Denmark used 97.000 kg of antibiotics (table 1, page 9). A large part of this difference can be assigned to the intensive use of tetracyclines in the Netherlands (225.000 kg vs 24.000 kg in Denmark). Tetracyclines are predominantly used in mass-medication of food animals.
Pigs, broilers and cattle are known to be the animal species to which most antibiotics for therapeutic use are administered. In table 2 antibiotic usages in both countries is related to

overall life weight production[2] of these species. It can be calculated that in the Netherlands 110 mg of antibiotics were used to produce 1 kg life weight (antibiotic growth promoters not included). In Denmark this was 33 mg. Further analysis of this difference per animal species is described here.

Pigs

In Denmark 75% (app. 72.000 kg) of the antibiotics, were used in pigs. In The Netherlands, FIDIN estimated over 1999 that 41% (133.000 kg) of antibiotics were used in pigs. Based on sampled data from veterinary practices in 1999 it was estimated that 63 % (202.000 kg) of the total amount of antibiotics was used in pigs.

Although exposure data of veterinary drugs are often expressed in kilogram of active substance, the actual exposure depends merely on the number of daily dosages an animal receives. Differences in potency of the drugs that are used can be used to explain the differences in sales and therefore have to be considered. In Denmark macrolides represent 20 % (15.000 kg) of total antibiotic usage in pigs whereas tetracyclines represent 30 % (22.000 kg). In the Netherlands this is only 1,3% (2.600 kg) for macrolides and as much as 56 % (113.000 kg) for tetracyclines (based on sampled data from veterinary practices). Macrolides are potent antibiotics requiring dosages 2-3 times lower than the tetracyclines. In the tetracycline group also a difference in usage patterns can be observed. In Denmark predominantly chlortetracycline is used, whereas in the Netherlands doxycycline and oxytetracycline are used. Despite this difference it can be estimated that the overall (mean) potency of the tetracyclines used in the Netherlands and in Denmark is comparable. So the main factor influencing the overall mean potency of the antibiotics used is the relatively high use of antibiotics other than tetracyclines and trimethoprim/sulphonamides in Denmark. This affects the overall mean potency of antibiotics used in Denmark related to The Netherlands, which is estimated to be 25% less than the overall potency of antibiotics used in pigs in The Netherlands.

Life weight production of pigs as reflected in table 3 was comparable between both countries in this period and can therefore not account for the difference. However, another factor is influencing these data. Piglets receive most antibiotics during weaning. Many pigs in The Netherlands are exported at a weight of about 25 kg from are finished to approximately 100 kg abroad. Life weight production data are based on the body weight at the time these animals are exported. So, when exported, these animals have already received a large part of their life quantity of antibiotics, but only count in meat production statistics for 25 kg. Once exported, during fattening they need less antibiotics. From the Netherlands 2,4 million pigs more are exported than from Denmark (4,0 million vs. 1,6 million). If these pigs had not been finished abroad but in The Netherlands, life weight production in pigs in the Netherlands had increased by 180 million kg (10%). Assuming that the same amount of antibiotics is used leads to a maximum correction of 10%.

Summarising: factors influencing a comparison of usage data in pigs are the different potencies of drugs used and the different export patterns between both countries. Based on estimates of FIDIN and sampled practices the use of antibiotics in pigs (corrected for potencies and export) was higher in the Netherlands than in Denmark.

Poultry

DANMAP 2002 reports a remarkably low consumption for Danish poultry. In over 15 million broilers only 62 kg of antibiotics were used in 2002. In The Netherlands, FIDIN estimated

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

over 1999 that 11% (34.000 kg) of antibiotics were used in poultry (broilers and layers). Based on sampled data from veterinary practices in 1999, it was estimated that 15% (47.000 kg) of the total amount of antibiotics are divided over 53 million broilers and (to a lesser extend) over 42 million laying hens.

Cattle

Most antibiotics in dairy cows are administered locally in the udder or in the uterus. Use of these locally administered drugs accounts for less than 1% of the total antibiotic mass (both in Denmark and in the Netherlands). Usage statistics of these antibiotics are not relevant for explaining the different consumption patterns in Denmark and the Netherlands.
For the Netherlands, FIDIN estimated over 1999 that 44% (140.000 kg) of antibiotics were used in cattle, about 99% of this in veal calves. A quantitative estimation based on sampled data from veterinary practices in 1999 could not be made but 140.000 kg was considered to be a high estimation. As table 1 indicates, in Denmark a veal calf industry, as known in the Netherlands is non-existent.
In DANMAP 2002 low consumption data for antibiotics are presented for Danish calves, only 1.427 kg is reported in calves. This figure has to be corrected, as 20% of usage in calves is used or distributed by veterinary practices and this is not included in the specified usage statistics of DANMAP. Therefore the total usage in calves in Denmark amounts to app. 1.800 kg. Total use in cattle in Denmark was estimated to be 10.000 kg

Comparison between Denmark and the Netherlands - Summary

Both Denmark and The Netherlands have restrictive antibiotic policies and are considered to be countries where antibiotic usage in livestock is low. Comparing sales data originating from Dutch pharmaceutical companies with consumption data from DANMAP leads to the conclusion that the mass of antibiotics used as medicines for therapeutic purposes related to the live weight produced is 3,3 times higher in the Netherlands. This corresponds with 300.000 kg antibiotics in 2002. The major part can be attributed to the intensive tetracycline use in the Netherlands. The data that are available from The Netherlands limit the possibilities for a detailed comparison.
Nevertheless, it can be concluded that differences in usage statistics between Denmark and the Netherlands are predominantly related to the use of antibiotics in the Dutch cattle. The difference in usage in cattle between both countries can mount up to 130.000 kg a year. The explanation for this difference is the existence of a large veal calf industry in the Netherlands accompanied by an intensive use of antibiotics. Moreover, more beef is produced in the Netherlands (table 2).
In Dutch pigs (depending on the estimation) 60.000 to 130.000 kg more antibiotics are used compared to Denmark. Livestock statistics from pigs seem to be comparable, however a correction due to export of young pigs from the Netherlands has to be made. The potency of drugs used in pigs in Denmark is higher than in the Netherlands but this also cannot entirely compensate for the difference in antibiotic mass used. Consequently the exposure of pigs to antibiotics is estimated to be higher in the Netherlands.
Dutch poultry uses approximately 34.000 to 47.000 kilograms whilst in Danish broiler production a negligible usage is reported.

Table 2 . Live weight production in the Netherlands and Denmark and sales of antibiotics in 2002.

	The Netherlands		Denmark	
	Live weight production (in kg x 10^6)	Antibiotic sales (in kg x 10^3)	Live weight production (in kg x 10^6)	Antibiotic sales (in kg x 10^3)
Cattle	434		304	
Veal calves	266		5	
Pigs	1.984		2.336	
Poultry	953		294	
TOTAL	3.637	402	2.939	97

Source: Dutch product board for livestock, meat and eggs (PVE), Danish Meat Board, FIDIN.

Table 3. Pig population statistics in the Netherlands and in Denmark in 1999 and 2002.

1999	**NL**		**DK**	
	Livestock (x10^6)	Live weight produced (kg x 10^6)	Livestock (x10^6)	Live weight produced (kg x 10^6)
Slaughter pigs (incl. piglets, weaners, pigs > 20 kg)	12,2	-	10,7	-
Sows and boars	1,4	-	1,3	-
pigs (total)	13,6	2.290	12,1	2.200
2002	**NL**		**DK**	
	Livestock (x10^6)	Live weight produced (kg x 10^6)	Livestock (x10^6)	Live weight produced (kg x 10^6)
Slaughter pigs (incl. piglets, weaners, pigs > 20 kg)	10,4	-	11,3	-
Sows and boars	1,2	-	1,4	-
pigs (total)	11,6	1.990	12,7	2.340

Source: Central Bureau of statistics (CBS), Dutch product board for livestock, meat and eggs (PVE), Danish meat board.

Appendix III. Materials and Methods

Salmonella spp.

A total of 7956 isolates were tested for antimicrobial resistance between 1999-2003 (Table 4). Human isolates (N=4279) 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= 635) and cattle, including calves (N=159) were sent to the RIVM by the regional Dutch Animal Health Services concerning approximately 80% clinical Salmonella infections. Those from chickens (broilers, including poultry products, N=762; layers, reproduction animals and eggs, N=419) concerned mainly nonclinical Salmonella infections derived from a diversity of monitoring programs on the farm, slaughterhouses and at retail. In 2001, 2002 and 2003 isolates from a diversity of other sources have been analysed as well (animal fodder and human food products; other animals from animal husbandry and pets, samples from the environment, etc.).

Table 4. Number of Salmonella isolates tested for susceptibility from 1999 – 2002 in the Netherlands.

	Total	1999	2000	2001	2002	2003
Human	4279	674	349	1056	862	1338
Pig	635	31	195	114	168	127
Cattle	159	18	28	56	33	24
Chicken (misc.)	488	0	20	154	142	172
Broilers (faeces/meat)	762	68	100	164	238	192
Layers/Repro/Eggs	419	93	86	80	69	91
Other sources	1214	22	22	331	353	486
Total	7956	906	800	1955	1865	2430

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 6 shows that quite substantial numbers are needed to indicate significant differences in resistance percentages less than 10%. However, resistance strongly depends on Salmonella type and many different types are involved; a cocktail of types that differs between sources and may differ between years. Moreover, low numbers tested and incidentally missed or selected types with rare antibiograms may influence the resulting resistance percentages. Finally the source definition in itself may be biased, as the reason for sending-in isolates, especially from cattle and pigs, is often unknown. This explains many of the irregularities between years.

Table 6. 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 *Campylobacter* spp. isolated from slaughter pigs and broilers

E. coli and *E. faecium*, and *Campylobacter* spp. were isolated from faecal samples taken from healthy animals at slaughter by the National Inspection Service for Livestock and Meat (RVV). Six pig- and six broiler slaughterhouses respectively, were randomly selected. These slaughterhouses were situated all over the country to eliminate potential regional differences. The sampling period in 2003 was January - April. At each slaughterhouse once daily from one animal a faecal sample (pigs) was taken aseptically, or the caeca collected (broilers). The vials were stored at 4 – 8°C until the next Monday, when they were sent to CIDC-Lelystad. At the Department of Bacteriology and TSEs the samples were directly 1:10 diluted in buffered peptone solution with 20% glycerol and stored at –20°C. *E. coli*, *E. faecium* and *Campylobacter* spp. were isolated directly after arrival of the samples at CIDC-Lelystad. For *E. coli* MacConkey agar and for *E. faecium* Slanetz and Bartley agar was inoculated with 50 µl of serial dilutions of the sample in saline with a spiral plater (*E. faecium*) or direct inoculation of the plates with cotton swabs (*E. coli*). A colony with typical morphology was subcultured to obtain a pure culture and stored at –80°C in buffered peptone water with 20% glycerol. *E. coli* was identified biochemically. The final identification of *E. faecium* was done with Polymerase Chain Reaction (PCR) as described by Dutka Malen in 1995.
For isolation of *Campylobacter* CCDA-agar with 32 µg/ml cefoperazone and 10 µg/ml amphotericin B to inhibit growth of Gram-negative bacteria and fungi, was directly inoculated with a cotton swab. All campylobacters were typed with PCR to the species level. Only *C. jejuni* and *C. coli* were tested for their susceptibility. All other spp. were excluded from the programme.

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

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

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

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

Pasteurella multocida and *Mannheimia haemolytica*

Bovine respiratory tract pathogens were isolated from specimens taken from diseased animals or at autopsy at the Animal Health Service in Deventer, The Netherlands. Species identification was done biochemically. No information was available on antibiotic usage in these animals. Probably, most of the animals were treated with antibiotics prior to sampling, indicating that these data are biased and may represent a worst-case scenario.
Isolates were sent to CIDC-Lelystad for MIC-determinations.

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

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

Susceptibility tests

Susceptibility was tested quantitatively with the broth micro dilution test with cation-adjusted Mueller Hinton broth according to NCCLS guidelines (M31-A2 and M7-A6). For broth micro dilution, microtitre trays were used with dehydrated dilution ranges of custom made panels of antibiotics. Trek Diagnostic Systems, in the UK, manufactured these microtitre trays. For the *Campylobacter* spp., after inoculation of the microtitre trays with 50 µl of a 200 fold diluted 0.5 McFarland suspensions in saline solution, the trays were incubated micro aerobically in a shaking incubator at 37°C for 48 hours. ATCC strains *E. coli* 25922 and *E. faecalis* 29212 were used daily to monitor the quality of the results. For quality control of the results of campylobacters, *C. jejuni* ATCC 33560 was used as control strain.
The MICs were defined as the lowest concentration without visible growth. Strains with MIC's higher than the MIC-breakpoints were considered resistant. Percentages of resistance were calculated. These were based on MIC-breakpoints listed in Table 9.

Table 9. MIC-breakpoints (µg/ml) used for susceptibility testing of bacteria. Isolates with MIC-values higher than those presented in this table are considered resistant.

	Salmonella spp. *E. coli*	*Campylobacter* spp.	*Enterococcus* spp.	*Pasteurella Mannheimia*	*E. coli (mastitis)*	*Streptococcus* spp.	*S. aureus.*	Coag. neg staphylococc	*Brachyspira* spp.
Penicillin	-	-	-	-	-	2	0,125	0,125	-
Oxacillin	-	-	-	-	-	-	2	0,25	-
Amoxicillin	16	16	8	16	16	-	-	-	-
Amox/clav. acid	16/8	-	8/4	16/8	16/8	8/4	4/2	4/2	-
Cephalothin	-	-	-	-	-	16	16	16	-
Cefuroxime	-	-	-	-	16	-	-	-	-
Cefoperazone	-	-	-	-	32	-	-	-	-
Ceftiofur	-	-	-	4	-	-	-	-	-
Cefquinome	-	-	-	4	4	-	-	-	-
Cefotaxime	1	-	-	-	-	-	-	-	-
Imipenem	1	-	-	-	-	-	-	-	-
Streptomycin	-	8	2000	-	32	-	16	16	-
Gentamicin	8	8	500	4	8	-	-	-	-
Kanamycin	-	-	-	-	16	-	16	16	-
Neomycin	16	8	-	16	16	-	16	16	-
Spectinomycin	-	-	-	64	-	-	-	-	-
Tetracycline	8	-	-	8	8	4	8	8	-
Doxycycline	4	4	8	-	-	-	-	-	-
Sulphamethoxazole	256	256	-	-	-	-	-	-	-
Trimethoprim	8	-	-	-	-	-	-	-	-
Trim/sulphamethoxazole	2/38	8/152	-	2/38	2/38	2/38	2/38	2/38	-
Nalidixic acid	16	16	-	-	-	-	-	-	-
Flumequine	4	-	-	4	-	-	-	-	-
Enrofloxacin	-	-	-	1	2	-	-	-	-
Ciprofloxacin	2	2	8	-	-	-	-	-	-
Chloramphenicol	16	16	16	16	-	-	-	-	-
Florfenicol	16	-	-	4	-	-	-	-	-
Nitrofurantoine	-	-	128	-	-	-	-	-	-
Vancomycin	-	-	16	-	-	-	-	-	-
Teicoplanin	-	-	16	-	-	-	-	-	-
Avilamycin	-	-	16	-	-	-	-	-	-
Bacitracin	-	-	128	-	-	-	-	-	-
Flavomycin	-	-	16	-	-	-	-	-	-
Quinu/dalfopristin	-	-	2	-	-	-	-	-	-
Virginiamycin	-	-	8	-	-	-	-	-	-
Erythromycin	-	16	4	-	-	0,5	4	4	-
Tylosin	-	-	-	-	-	-	-	-	16
Tilmicosin	-	-	32	16	-	-	-	-	-
Lincomycin	-	-	-	-	-	4	4	4	-
Pirlimycin	-	-	-	-	-	2	2	2	-
Tiamulin	-	-	-	-	-	-	-	-	2
Metronidazole	-	4	-	-	-	-	-	-	-
Salinomycin	-	-	4	-	-	-	-	-	-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