MARAN 2002

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

This report is published under the acronym *MARAN-2002* by VANTURES, the Veterinary Antibiotic Usage and Resistance Surveillance Working Group. The information presented in *MARAN-2002* is based on a collation of data from ongoing surveillance systems on the use of antimicrobial agents in animal husbandry and the development of antimicrobial resistance in bacteria of animal origin and of relevance to public health. All these surveillance systems already existed in 2002. The studio of the Dutch Food and Consumer Product Safety Authority (VWA) in The Hague The Netherlands produced this document.
MARAN-2002 can be ordered from the secretariat of the VWA, p/a CentreCourt, P.O.Box 19506, 2500 CM The Hague, The Netherlands. *MARAN* is also available from the websites of the VWA: www.vwa.nl, and CIDC-Lelystad: www.cidc-lelystad.nl.
The citation is: *MARAN-2002* - Monitoring of Antimicrobial Resistance And Antibiotic Usage in Animals in The Netherlands In 2002

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

Contents

Summary

MARAN 2002 presents data on resistance in zoonotic and commensal bacteria of animal origin, and data on usage of antimicrobials in food producing animals.
Data from 2002 in the current report indicate a slow overall increase in **flumequine** resistance (all flumequine-resistant strains demonstrate decreased susceptibility to ciprofloxacin) in salmonella's in The Netherlands. This increase is serotype specific. In *S.* Typhimurium DT104 and ft 507, the flumequine resistance levels were low and stable. In *S.* Java, however, a very rapid increase in flumequine resistance was observed from 0% in 2000 to 35% in 2002 in strains from broilers. In *S.* Enteritidis pt1 relatively high levels of flumequine-resistance were observed pt 1 isolated from human patients. Eggs imported from Spain are documented as the source of these strains. **Fluoroquinolone**-resistance in *C. jejuni* strains was stable around 40% in poultry. The resistance percentages in human clinical isolates have slowly increased in the last decade from approximately 20% in 1992 to 32% in 2002. However travel abroad was considered an important risk factor for the acquisition of fluoroquinolone resistant strains.
Resistance to **cefotaxime** (ESBL-positive) was found; both in human and poultry salmonella's and in *E. coli* strains from broilers. Resistance to **vancomycin** in *E. faecium* remained stable at low levels both in broilers and in pigs in spite of the fact that glycopeptides have not been used for years. Although macrolide resistance in *E. faecium* strains from pigs seemed to further decrease, resistance to the other growth promoters stabilized after the initial decrease when these products were banned in 1999.

The therapeutic use of antibiotics in animals in The Netherlands has increased annually since 1990, while the demographic data show that livestock has decreased. The main antibiotic classes used in food producing animals were tetracyclines and trimethoprim-sulphonamide combinations. The overall consumption of therapeutically used antibiotics in The Netherlands in food-animals was substantially higher than in Denmark or the UK.
It can be concluded that this first coordinated report of the surveillance activities on antimicrobial consumption of veterinary licensed drugs and resistance in animal bacteria has provided very useful data. Observed trends in resistance can partly be attributed to consumption patterns in food animals. Moreover this programme provides data on antimicrobial resistance in bacteria of public health conceren as requested in the new EU zoonoses directive.

Based on the results of this report it can be recommended that:
- this coordinated monitoring programme should be continued and should report annually;
- the report of consumption data should be standardised at the international level to improve comparability of the data;
- the DDD-analyses of detailed usage data on practitioners-, or farm level should be improved regarding the representativeness of the selection of practitioners and farms included in the analysis;
- although the major food-animal species in The Netherlands (poultry and pigs) are well examined in the present programme, resistance data on strains from cattle (dairy and meat) should be improved;
- Measures should be taken to reduce the consumption of antimicrobial agents;
- trends in increase of quinolone resistance in Salmonella, Campylobacter and *E. coli* in food animals and in humans warrant optimisation of prudent use policies;
- imported food products, including vegetables irrigated with faecal contaminated water, should be included in the monitoring programme.

Samenvatting

MARAN 2002 bevat gegevens over resistentie in zoönotische en commensale bacteriën van dierlijke oorsprong en gegevens over het gebruik van antimicrobiële stoffen in voedselproducerende dieren.
De data uit 2002 in dit rapport geven aan dat er een langzame toename in resistentie tegen **flumequine** (deze stammen zijn ook allemaal minder gevoelig voor ciprofloxacin) waarneembaar is in salmonella 's geïsoleerd in Nederland. De resistentie toename is serotype specifiek. In *S.* Typhimurium DT104 and ft 507, waren de flumequine resistentieniveaus laag en stabiel. In *S.* Java uit slachtkuikens daarentegen werd van 2000 tot 2003 een snelle toename in flumequine resistentie waargenomen (0% - 35%). In humane klinische isolaten van *S.* Enteritidis pt 1 werd relatief vaak flumequine resistentie waargenomen. Deze stammen bleken geïmporteerd uit Spanje via gecontamineerde eieren.
Resistentie tegen **cefotaxime** (ESBL-positief) werd zowel in humane salmonella's als in salmonella's uit pluimvee waargenomen. Ook in commensale *E. coli*'s uit slachtkuikens komt cefotaxime resistentie voor hetgeen een aanwijzing voor mogelijke selectie van deze resistentiedeterminant door b.v gebruik van amoxicilline.
Hoewel avoparcine als groeibevorderaar al in 1997 werd verboden, blijf resistentie tegen **vancomycine** in *E. faecium* stabiel op een laag niveau in slachtkuikens en slachtvarkens. Ook voor de overige groeibevorderaars, m.u.v. de tylosine waarvoor een verdere afname in resistentie werd waargenomen, bleven de resistentiepercentages stabiel.
Het therapeutische gebruik van antibiotica in dieren in Nederland is toegenomen vanaf 1990, terwijl de totale aantallen landbouwhuisdieren zijn afgenomen. De meest gebruikte antibiotica klassen in dieren waren de tetracyclinen en trimethoprim/sulfonamiden combinaties. In vergelijking met het Verenigd Koninkrijk en Denemarken worden in Nederland beduidend meer antibiotica gebruikt in voedselproducerende dieren.
Wij concluderen dat het eerste gecoördineerde rapport van de surveillance activiteiten van antimicrobiële resistentie en consumptie van antibiotica zeer waardevolle gegevens heeft opgeleverd. Waargenomen trends in resistentie kunnen deels gerelateerd worden aan gebruikspatronen. Bovendien sluit dit monitoringsprogramma grotendeels aan op de eisen van de nieuwe zoönosen richtlijn.
Gebaseerd op de resultaten van dit rapport worden de volgende aanbevelingen gedaan:

- dit gecoördineerde monitoringsprogramma dient te worden gecontinueerd en jaarlijks rapporteren;
- de gebruiksgegevens dienen op internationaal niveau te worden gestandaardiseerd om de vergelijkbaarheid te vergroten;
- de DDD-analyses van gedetailleerde gebruiksgegevens van dierenartsenpraktijken en boerderijen dienen te worden verbeterd t.a.v de representativiteit van de selectie van dierenartsen en bedrijven in de steekproeven;
- hoewel de belangrijkste soorten Nederlandse voedselproducerende dieren uitgebreid onderzocht zijn in het huidige programma, dienen de gegevens van runderen (vlees-, en melkvee) te worden verbeterd;
- maatregelen dienen te worden genomen om de hoeveelheid van gebruikte antibiotica in voedselproducerende dieren te verminderen;
- trends in toename van quinolonen resistentie in Salmonella, Campylobacter en *E. coli* in voedselproducerende dieren nopen tot een aanscherping van het antibioticumbeleid;
- geïmporteerde producten van voedselproducerende dieren, inclusief groente wat tijdens de groei bevloeid is met fecaal gecontamineerd water, dient te worden betrokken in het monitoringsprogramma.

I Introduction

Antibiotic resistance development is more and more of public health concern, because it is increasing. If no action is undertaken the moment may arrive that physicians will be left without tools to treat certain infectious diseases. The ministries of Agriculture and Public Health perceived this trend, and each implemented programs to prevent further increase. One of the actions was to monitor the antimicrobial resistance development of pathogens both in humans and in the veterinary and agricultural sector.
The Interdepartmental Working Group on Antimicrobial resistance of the Ministry of Public Health Welfare and Sports and Ministry of Agriculture Nature and Food Quality coordinates monitoring of antimicrobial usage and resistance in the Netherlands. This Working Group coordinates the activities of two subgroups. One subgroup is SWAB (the Dutch foundation of the Working Party on Antibiotic Policy), which recently published its first report, Nethmap-2003, on antibiotic usage and resistance data among medically important bacteria in The Netherlands, excluding food-borne pathogens. The second subgroup is VANTURES (Veterinary Antibiotic Usage and Resistance Surveillance). This working group was installed in May 2003 with the assignment to organise and report the surveillance data of veterinary antibiotic usage and resistance in The Netherlands on a yearly basis. A preliminary report on the monitoring of antimicrobial resistance in bacteria of animal origin up until 2002, MARAN-2001, was published in March 2003. The veterinary monitoring programme started in 1999. It is co-ordinated by the Central Institute for Animal Disease Control (CIDC-Lelystad) in Lelystad, The Netherlands and carried out in co-operation with the National Institute of Public Health and the Environment (RIVM) in Bilthoven, the Faculty of Veterinary Medicine in Utrecht , the Inspectorate for Health Protection and Veterinary Public Health (VWA-KvW) in Zutphen and The Food and Consumer Product Safety Authority (VWA) in The Hague, the Netherlands. The programme is aimed at potential zoonotic aspects of antimicrobial resistance in animal husbandry by transfer of resistant strains or resistance genes in bacteria from animals to humans. The most important way of transfer is considered to be the food chain. Direct contact may be important for specific animal owners but is considered to be a low risk for public health in general. For this reason only the major food animal species (broilers and slaughter pigs) are included in the programme.
Because of the zoonotic aspect the bacterial species focussed on as vehicles of resistance determinants are the zoonotic food-borne pathogens *Salmonella* spp. *Campylobacter* spp., and Shigella toxin producing *Escherichia coli* (STEC). Moreover indicator organisms of the normal bacterial flora of the intestinal tract are included. *E. coli* as indicator for the Gram-negative flora and *Enterococcus faecium* as indicator for the Gram-positive flora, both important sources of resistance determinants that could be transferred to human bacteria.
The inclusion of animal pathogens is of indirect importance for public health, but still very relevant. These pathogens usually represent a worst-case scenario because of sampling bias. Therefore these strains can be used for early warning purposes regarding the detection of new emerging resistances.

The purposes of this antimicrobial resistance monitoring programme are:

- detection of the emergence of new resistance phenotypes;
- determination of trends in resistance in time;
- detection of potential public health risks.

II Usage of antibiotics in animal husbandry in the Netherlands

Antibiotics are administered to food-producing animals as feed additives or as therapeutic agents. If antibiotics are used as a feed additive to promote efficient uptake (lower cost price of meat and less manure production), they are referred to as antimicrobial growth promoters. Antibiotics that are administered as feed additive for their activity against coccidia, are referred to as coccidiostats.

Usage of antimicrobial growth promoters (AGPs) and coccidiostats

Manufacturing, distributing and selling animal feed containing AGPs is in the hands of the feed industry. Therefore, sales figures of AGPs cannot easily be obtained and there is only a limited knowledge about the use of antibiotics as a feed additive in The Netherlands.
In the past estimates were made about the use of AGPs. One of them was published by FEDESA (the former association of veterinary medicine manufacturers in Europe, now named IFAH). The sales of AGPs in the EU in 1997 was estimated to be 1,600 x 10^3 kg active substance and the sales of antimicrobial medicines for veterinary use was estimated to be 3,500 x 10^3 kg active substance. In 1999 these figures were estimated to be 800 x 10^3 and 3,900 x 10^3 kg respectively. The relative share of additives was therefore halved after the the partial prohibition on growth promoters (see below).
Also for The Netherlands estimates on the use of AGPs were made. In 1998, 250 x 10^3 kg of antibiotics were used as AGP in the Netherlands, while in that year 300 x 10^3 kg of antibiotics (active substance) was used for therapeutic purposes (Fig. 1).
Since cross resistance occurs between antibiotics used as AGP and antibiotics used therapeutically for animals and humans, the use of antibiotics as AGP was put under pressure. Since 1999 only a 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. In 2006, all antibiotics used as AGP will be prohibited in EU. Bearing this in mind, setting up a monitoring system for use of AGPs has a low priority. No data are available for the use of coccidiostats. These data can only be provided by the feed industry.

Usage of antibiotics as medicines for therapeutic purposes.

More data are available on amounts of antimicrobial agents for therapeutic use in food-producing animals in The Netherlands. Initiatives carried out to measure exposition of animals to veterinary antibiotics, are described below. Data for companion animals were estimated to amount to approximately 1% of the total use of antibiotics in animals.

Total sales, provided by the pharmaceutical industry

Since 1990 the therapeutic use of antibiotics in the Netherlands is monitored. This is based on total sales data generously provided by the Dutch veterinary pharmaceutical industry.

Table 1. Total sales in food-producing animals in 2002 in The Netherlands (Source: FIDIN)

	Active substance (kg x 1000)	% of total use
Beta-lactams/cephalosporins	40	10 %
Tetracyclines	225	56 %
Macrolides	20	5 %
Aminoglycosides	10	2 %
(Fluoro)quinolones	6	1 %
Trim/Sulpha's	94	23 %
Others	11	3 %
Total	402	100 %

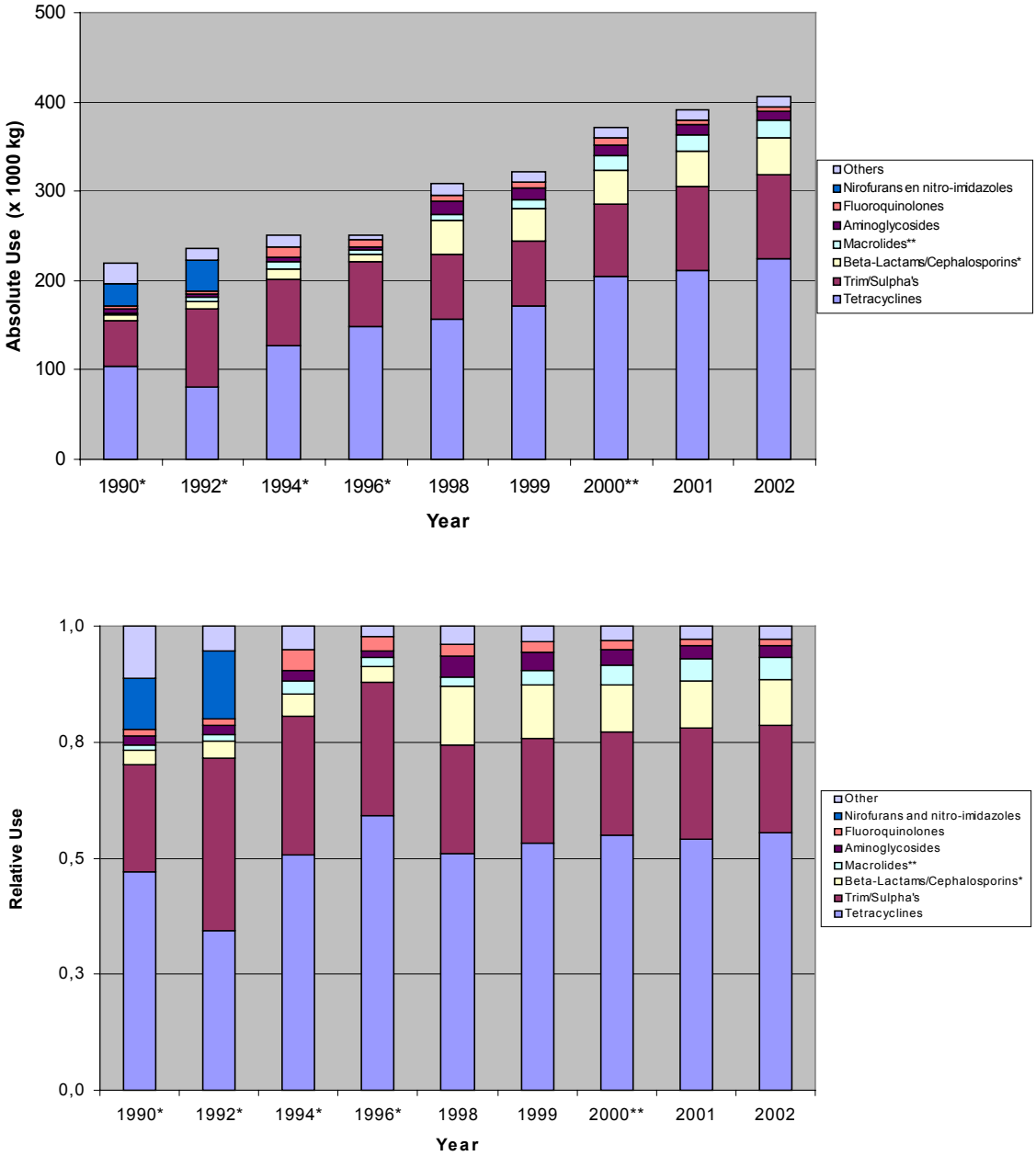
In 2002 tetracyclines and trimethoprim/sulphonamide combinations represented 79% of the total sales of antimicrobials, expressed in kilograms, for food producing animals. Quinolone-sales (in kg) represented 1% of total sales. However, quinolones have a high potency and the relative number of doses will thus be more than 1 %. Altogether, more than 400.000 kg of veterinary antimicrobial drugs were sold in 2002.
Sales from 1990 to 2002, expressed in kg, are summarized in Figure 1 including the relative contribution of each therapeutic group. Some of the changes are related to the way data were sampled and analysed. The data from 1990 to 1996 are based on a publication by Pijpers et al. However data concerning 1990 and 1992 were first published by the Dutch Veterinary Antibiotics Policy Working Party. Pijpers et al reviewed this data and produced new data for 1994 and 1996. Unfortunately he was not able to calculate the use of antibiotics used for individual medication. The data from 1990 to 1996 presented in figure 1 are therefore exclusively based on group medication in food animals. This implicates total use in this period to be 1,20 – 1,25 times higher. More specific, as beta-lactams are mainly used in individual medication, total use of beta-lactams will have been 2 times higher.
Since 1998, the association of manufacturers and importers of medicines for veterinary use in the Netherlands (FIDIN) reports total sales figures. Data are obtained from total sales numbers (individual and group medication) reported by all individual FIDIN members. FIDIN-members do not represent the total market in the Netherlands, but more than 95% in 2003. In 1999 products were taken over from non-FIDIN members and this has caused extra sales of 5% in 2000 compared to 1999.
Taking into account the confounders mentioned, the total sales of antimicrobial drugs for therapeutic use have risen from 275.000 kg in 1990 to 406.000 kg in 2003 (147% increase). Changes in livestock statistics (Table 2) cannot account for this difference. The increase may be attributed to new infectious diseases like circo virusses and *Lawsonia intracellularis* infections in pigs and scaling-up of food-animal farms.

Table2. Livestock population statistics (1992 and 2002) in The Netherlands (Source: CBS).

	1992	2002
Pigs (total)	**14.160.000**	**11.648.000**
Piglets	5.270.000	4.745.000
Pigs for fattening (>20kg)	7.145.000	5.591.000
Sows and boars	1.745.000	1.312.000
Chicken (total)	**99.389.000**	**101.052.000**
Broilers	46.525.000	54.660.000
Broilers, breeding females	7.844.000	7.503.000
Laying hens	44.989.000	38.889.000
Other poultry (ducks, turkey)	2.842.000	2.599.000
Cattle (total)	**4.920.000**	**3.858.000**
Fattening calves	638.000	713.000
Dairy cows	3.490.000	2.754.000
Cows for fattening and grazing	792.000	392.000

In general the relative contribution of different antibiotic-classes to total sales has remained stable. Sales of fluoroquinolones include flumequin and have risen from 1990 until 1996 but subsequently remained on a constant level. Nitrofurans and imidazoles accounted in the early 1990's for 10% of the total sales. Since use of these antibiotics is not allowed in food producing animals they have been withdrawn from the market. From 1998 until 2002 the use of beta lactams and aminoglycosides seems to have increased, but this effect is mainly due to the inclusion in the data of individually used antibiotics (by injection). The use of macrolides has increased from 6,000 kg in 1998 to 20,000 kg in 2002, which may have a relation with the ban of macrolides as AGPs.

Figure 1. Absolute and relative use of antibiotics for therapeutic use (active ingredient x 1000 kg) in The Netherlands from 1990 – 2002 (Source: FIDIN)



Until 1996 excl. cephalosporins, and until 1996 incl. lincosamides
The data until 1996 only represent the consumption as mass-medication in food-animals. After 1996 it represents the total consumption in The Netherlands

Sales data provided by veterinary practices

To obtain more detailed data concerning therapeutic use of antibiotics, records from veterinary practices can be used. In order to determine the usefulness of these data, the Pharmacy at the Faculty of Veterinary Medicine in Utrecht has undertaken a pilot-study to measure usage of antimicrobial medicines in farm-animals in the Netherlands based upon this data source. This study used electronically stored administrative data from veterinary practices. Included in the study were 24 practices, they represented 3% of the total pig population in the Netherlands and 1,2 % of total poultry population. Exposure of animals to antibiotics administered orally or by injection (systemic use) is expressed as number of Defined Daily Dosages (DDDs) administered per 1000 animal days. Local exposure in cattle (intra-mammary and intra-uterine therapy) is expressed as number of administrations per 1000 animals.

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

Numerator

Exposure data of veterinary drugs are often expressed in kilogram of active substance. In order not to underestimate the use of high potency drugs, the number of doses is preferred as a unit of measurement. To calculate the number of DDDs (meaning the number of daily doses the population at risk has been exposed to), a *defined daily dose* (DDD) for each active substance, animal species and administration route has to be established.
In human medicine DDDs are assigned by the WHO International Working Group for Drug Statistics Methodology, allowing for comparisons of medicine use. In human medicine, DDD is defined as the assumed average maintenance dose per day for the medicine used in its main indication in adults. Such international recognized DDDs are not yet assigned for medicines for veterinary use. Grave et al, introduced tentative DDDcow as a unit to measure the consumption of antimicrobial medicines in bovine mastitis. Because there is yet no international consensus concerning the value of these DDDs, they can be designated as local DDDs.

Denominator

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

By using the number of DDDs for each individual animal over a certain period of time as a criterion for exposure to antibiotics, it is possible to relate the extent of antibiotic use among different types of food-producing animals and between food-producing animals and humans.

In 2000, the human population in the Netherlands (outside hospital) was prescribed an antibiotic 0.4 times per year. A prescription consisted of an average of 8.6 DDDs. Dutch people outside hospital therefore receive antibiotics for 3.2 days a year. In other words, the exposure to antibiotics outside hospital is 9.4 DDDs per 1000 days. In 1996 in Dutch hospitals, 372 DDDs per 1000 bed days were given. It can be concluded that people outside hospital are less frequently exposed to antibiotics than poultry or pigs. However, for patients in hospitals, the exposure is much larger.

In Table 3 exposure to antibiotics for systemic use (oral and injection) in pigs and poultry is presented. It was found that 48 DDDs per 1000 pig days were administered in 1999. Eighty percent of the doses for pigs (38 DDDs per 1000 pig days) were given as group medication. Of these antibiotic doses given to pigs, 67% consisted of a tetracycline, trimethoprim or a sulphonamide. For pigs quinolones and fluoroquinolones were hardly prescribed at all. Poultry were administered 45 DDDs per 1000 poultry days. Virtually all the doses were given as group medication. Eleven percent of the doses consisted of quinolones and fluoroquinolones.

Table 3. Exposure to antibiotics (number of DDDs per 1000 animal days) in pigs and poultry in 1999 (data from 24 veterinary practices) in The Netherlands (Source: Faculty of Veterinary Medicine, Utrecht).

	Group Medication		Individual Medication	
	Pigs	**Poultry**	**Pigs**	**Poultry**
Broad spectrum beta-lactams	0.4	3.2	1.1	0.0
Narrow spectrum beta-lactams	0.1	2.1	4.2	0.0
Cephalosporins	0.0	0.0	0.0	0.0
Tetracyclines	17.8	25.8	0.8	0.0
Aminoglycosides	0.3	0.4	2.5	0.0
Spectinomycin	0.1	0.0	0.0	0.0
Fluoroquinolones	0.0	2.3	0.1	0.0
Quinolones (flumequin)	0.0	2.6	0.0	0.0
Linco/Clindamycin	0.3	0.0	0.0	0.0
Macrolides	1.2	0.1	0.1	0.0
Trimethoprim	6.5	3.7	0.5	0.0
Sulphonamides	6.8	4.4	0.3	0.0
Polymyxins	4.4	0.4	0.3	0.0
Tiamulin	0.5	0.0	0.0	0.0
Various	0.0	0.0	0.0	0.03
TOTAL	**38**	**45**	**10**	**0,03**

Local exposure to intra-mammary and intra-uterine administered antibiotic preparations in dairy cattle of all ages was also measured (Table 4). In Table 5 these data are subdivided into therapeutic classes. The added number of doses can surpass the number in table 4, for each administration form may contain more than one antibiotic.
It was found that for 1000 animals, almost 900 lactation preparations were used. Presuming a mean treatment period of four days, 5,5 % of the udders of dairy cattle (total of young and milkingcows) received a therapeutic antibiotic treatment in 1999. Aminoglycosides (neomycin) and benzylpenicillin were most frequently used.
In 1999 almost 1200 dry-cow preparations were administered to 1000 cattle (4000 quarters) (Table 4). Per quarter, one dose was administered meaning that 30% of all udders in the dairy

cattle population (total of young and milking cows) received a prophylactic antibiotic treatment. Small spectrum beta-lactams were most frequently used.
Annually 100 intra-uterine dosage forms were administered per 1000 animals, mainly containing tetracyclines.

Table 4. Number of administrations (preparations for intra-mammary and intra-uterine therapy) in dairy cattle per 1000 animals in 1999 in The Netherlands (Source: Faculty of Veterinary Medicine)

Intra-mammary therapy during lactation	861
Intra-mammary dry cow therapy	1196
Intra-uterine therapy	102

Table 5. Number of administrations per active ingredient in intra-mammary and intra-uterine preparations in dairy cattle (young and lactating, 1999) per 1000 animals (Source: Faculty of Veterinary Medicine, Utrecht)

	Intra-mammary therapy during lactation	Intra-mammary dry cow therapy	Intra-uterine therapy
Benzylpenicillin	348	419	0
Cloxacillin and nafcillin	17	1053	0
Broad spectrum beta-lactams	160	675	0
Amoxicillin/Clavulanic acid	143	0	0
Cephalosporins	108	0	22
Aminoglycosides	590	316	0
Tetracyclines	162	0	78
Other	393	7	0

Using sales data from veterinary practices, off-label use of antibiotics could also be observed. Although lincomycine is licensed for use in pigs and poultry as a water-soluble powder, 85% was used in cattle. Presumably these antimicrobials were used in foot bathes to treat or prevent foot diseases like Morbus Mortellaro.
During the study, supplying accurate data on the number of animals proved to be difficult for participating veterinary practices. Often these data were not precisely known and have been estimated. The quality of this information is therefore difficult to check.

Purchase provided by farmers

By using sales data provided by veterinary practices it was possible to gain an insight into the use of antibiotics at animal species level. Nevertheless, for identification of risks, more detailed exposition data should also be used. The Agricultural Economics Research Institute (LEI) is an institute in the Netherlands for social and economic research on agriculture, horticulture, fisheries, forestry and rural areas. LEI has the 'Farm Accountancy Data Network' (FADN) at its disposal. Various data from a random sample of agricultural and horticultural holdings are stored in this network. Original and main objective of the FADN is to get representative information on the developments in income of Dutch farmers. In recent years extra aims were added to provide insight in the performance of the farmers concerning environmental and animal health issues. Based on this network a project upon the economics of veterinary services and medicines on pig- poultry and dairy farms was performed.

Economic data concerning veterinary medicines, originating from farm accountancies were obtained from a sample of the farms of FADN. LEI has also detailed information regarding the exposed population. This data-combination was analysed in cooperation with the Pharmacy of the Faculty of Veterinary Medicine in Utrecht.

Most of the trimethoprim/sulphonamides and broad-spectrum beta-lactams were used in pig reproduction facilities, probably used in weaning piglets (Table 6). Macrolides on the contrary were mainly used in meat production. Exposure to antibiotics was concentrated in breeding facilities rather than in fattening farms.
These results are comparable to those from veterinary practices although use of trimethoprim-sulphonamide combinations seems to be higher in the results obtained from LEI. Those differences are probably related to the size of the provisional sample; only 0,5% of the total Dutch pig population was included in the current LEI sample.

Table 6. Exposure (Number of daily doses per 1000 animal days) to antibiotics in three types of pig farms (data from farms) in the Netherlands in 1999 and 2000 (Source: LEI and Faculty of Veterinary Medicine).

	Closed facilities (breeding and fattening)	Breeding (sows and piglets)	Fattening facilities
Group medication			
Broad spectrum beta-lactams	0,1	1,6	0,2
Tetracyclines	15,5	15,0	11,4
Macrolides	3,4	2,3	5,7
Lincosamides	0,3	0,5	0,0
Spectinomycin	0,3	0,5	0,0
Sulphonamides	20,9	22,4	9,2
Trimethoprim	20,9	22,4	9,2
Polymyxins	2,7	8,2	1,4
TOTAL	**64,1**	**72,9**	**37,2**
Individual medication			
Broad spectrum beta-lactams	0,9	2,4	0,7
Small spectrum beta-lactams	2,8	6,4	2,5
Tetracyclines	0,1	0,5	0,6
Aminoglycosides	1,1	2,3	1,6
Fluorquinolones	0,1	0,1	0,0
Macrolides	0,1	0,0	0,1
Lincosamides	0,0	0,0	0,0
Sulphonamides	0,3	1,0	0,0
Trimethoprim	0,3	1,0	0,0
Polymyxins	0,0	0,8	0,0
TOTAL	**5,7**	**14,5**	**5,6**

Conclusion

Three projects concerning the exposition of animals to veterinary drugs are carried out in The Netherlands. Major differences between the studies are the data-sources used, the units of measurement used to report the results and the level of detail of the exposed animal population.
The data provided by the veterinary pharmaceutical industry gives information ontherapeutic use of veterinary antibiotics in the Netherlands as a whole. Using these data, trends in time can be observed. These data are relatively easy to obtain. Unfortunately, it is not possible to gain an insight in the use of antibiotics at animal species level as 70% of the active substances is used in more than one animal species. From the sales data presented, no subdivision can be made between fluoroquinolones and flumequin or between small-spectrum beta-lactams, beta-lactams with extended spectrum and cephalosporins. To identify risks concerning the use of antimicrobials more detailed data are needed. Exposure data should not be expressed in kg but in number of doses. In addition to this it is essential to have detailed information concerning the exposed population.
Therefore antibiotic use was measured at the level of the veterinary practitioner. Measurement of usage on animal species level was not a problem, but it was difficult to obtain a reliable estimation of the exposed population. Furthermore, it was not possible to measure the use of antibiotics at production level (sow, piglet, fattening pig).
On the farm level, detailed information on livestock is present, allowing detailed risk identification. However, the results provided by LEI presented in this report were based on a relative small sample of farms. It will be possible to extend the sample to all of the livestock farms in the FADN-network. By sampling data on a practitioners- or farm level it was not possible to obtain data on use of antimicrobials in veal calves.
The combination of overall data from the industry (expressed in kilogram) and sampled data from farms (in number of doses per animal year) may provide very useful information for policy making in the future.

III Resistance data

In this chapter susceptibility test results are presented as determined in 2002 of 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,* the porcine enteric pathogens *Brachyspira hyodysenteriae,* 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.
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 2002, like in former years, *S.* Typhimurium and *S.* Enteritidis were by far the most prevalent 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 exists in prevalence of *Salmonella* spp. between broilers and layers. In broilers *S.* Paratyphi B var. Java (*S.* Java) and *S.* Infantis, and in layers *S.* Enteritidis and *S.* Infantis were the most prevalent serotypes.

Table 1. Most prevalent *Salmonella* serotypes isolated in 2002 from humans, pigs, poultry, broilers and layers[1].

Salmonella serotype		% of total isolated in 2002					
		Humans	Pigs	Catlle	Poultry	Broilers	Layers
Total number		1588	570	175	2043	940	184
Typhimurium		31,9	45,4	32,6	2,6	2,7	3,8
	DT 104	10,3	13,0	22,9	1,1	0,9	2,2
	ft 507	4,0	6,1	0,6	0,2	0,2	0,0
Enteritidis		44,5	0,2	0,0	6,8	1,9	41,8
Paratyphi B v Java		0,4	0,0	0,0	52,3	57,3	5,4
Dublin		0,3	0,4	53,1	0,0	0,0	0,0
Infantis		2,0	4,6	1,1	12,6	14,8	16,8
Bovismorbificans		0,9	13,0	0,0	0,0	0,0	0,0
Derby		0,7	9,6	1,1	0,2	0,1	1,1
Virchow		1,9	0,0	0,0	3,1	2,4	7,1
Brandenburg		2,1	6,3	0,6	0,4	0,6	0,0
Mbandaka		0,3	0,0	0,0	6,9	6,4	1,6
Livingstone		0,0	2,8	0,0	2,0	2,3	2,2
Indiana		0,1	0,0	0,0	2,7	1,9	4,9
Manhattan		0,9	4,2	0,0	0,2	0,3	0,0
Agona		0,5	0,5	0,0	1,4	2,6	0,5
Goldcoast		0,8	3,2	0,0	0,0	0,0	0,0
London		0,4	3,3	0,0	0,1	0,0	0,0
Heidelberg		0,4	0,0	0,0	0,5	0,3	2,7
Senftenberg		0,1	0,2	0,0	1,0	0,7	2,2
Panama		0,3	0,7	1,1	0,0	0,0	0,5
Kentucky		0,7	0,0	1,1	0,0	0,1	0,0
Hadar		1,1	0,2	0,0	0,3	0,5	0,0
Bareilly		0,3	0,0	0,0	1,2	0,9	0,5
Typhi,Paratyphi A,B		1,5	--	--	--	--	--
Newport		0,5	0,0	0,0	0,0	0,1	0,0
Other serotypes		7,4	5,4	9,3	5,7	4,1	8,9

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

[1] Report on trends and sources of zoonotic agents in the EU, 2002, The Netherlands

Table 2. MIC distribution (in %) for all salmonella's (N = 1865) tested for antibiotic susceptibility in 2002.

	MIC-distribution (µg/ml)															
Total	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**R%**
Amoxicillin	-	-	-	-	-	31,4	49,3	1,4	0,1	0,1	-	0,3	17,5	-	-	-	17,8
Cefotaxime	-	-	-	90,6	7,5	1,0	0,1	-	0,1	0,1	0,6	-	-	-	-	-	0,9
Cefuroxime	-	-	-	-	-	-	-	-	-	-	98,7	0,8	0,1	0,4	-	-	1,3
Imipenem	-	-	-	78,8	20,2	1,1	-	-	-	-	-	-	-	-	-	-	0,0
Gentamicin	-	-	-	-	43,1	47,2	8,1	0,6	0,1	0,1	0,3	0,1	0,4	-	-	-	0,8
Neomycin	-	-	-	-	-	-	74,8	21,8	2,8	0,2	0,1	-	0,2	0,1	0,1	-	0,3
Doxycycline	-	-	-	-	-	0,1	5,1	45,4	32,2	8,3	3,7	3,1	2,1	-	-	-	17,1
Trim/sulpha	-	-	-	-	84,5	1,8	0,3	0,2	0,3	0,2	0,1	0,1	12,7	-	-	-	12,8
Trimethoprim	-	-	-	-	-	79,0	4,8	1,7	0,9	0,1	0,1	-	-	13,4	-	-	13,4
Ciprofloxacin	-	52,1	38,1	2,1	4,6	2,5	0,5	-	-	0,1	0,1	-	-	-	-	-	0,1
Flumequine	-	-	-	-	-	46,2	41,3	2,9	0,6	3,2	4,0	1,4	0,3	0,1	-	-	9,0
Chloramphenicol	-	-	-	-	-	-	-	0,8	32,4	56,3	3,4	-	0,1	2,1	4,9	-	7,1
Florfenicol	-	-	-	-	-	-	-	4,8	73,8	14,5	1,9	4,2	0,3	0,5	-	-	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. The vertical bars indicate the breakpoints.

Table 2 presents MIC-distributions and resistance percentages of all salmonella's tested. Highest levels of resistance were observed for amoxicillin, doxycycline, trimethoprim and its combination with sulphamethoxazole. Also resistance to "newer" antibiotics exists. Fifteen cefotaxime resistant strains were found, ten originated from poultry and four from human patients. These isolates belonged to the following serotypes: 4 *S.* Bareilly and 3 *S.* Paratyphi B var. Java from poultry, and 3 *S.* Typhimurium ft 507 (Dutch phage typing system), two of them isolated from human patients. The majority of these strains were phenotypically suspected of extended spectrum beta-lactamase's (ESBL's). Fifteen gentamicin resistant strains, and eight neomycin resistant strains were found, which is similar to numbers found in 2001. Two ciprofloxacin resistant strains (MIC's > 4 µg/ml) were isolated from human patients (one *S.* Kentucky and one travel related *S.* Paratyphi A), and 168 flumequine resistant strains were found. These strains all showed reduced susceptibility to ciprofloxacin (MIC ≥ 0,125 µg/ml). Two multiresistant *S.* Newport strains were found, one isolated from poultry and one from a human patient. The resistance profiles of these strains were Amoxicillin/Gentamicin/Neomycin/Doxycycline/Chloramphenicol and Amoxicillin/Gentamicin/Doxycycline/Trimethoprim/Flumequin/Chloramphenicol/Florfenicol , respectively. Both strains were susceptible to cefotaxime and cefuroxime and therefore phenotypically different from the clone spreading in the US.

Table 3. Resistance percentages of the most prevalent *Salmonella* serotypes isolated in The Netherlands in 2002 including *S.* Typhimurium phage types DT104 and ft507.

	Typhimurium (N = 443)	DT104 (N = 129)	ft 507 (N = 60)	Enteritidis (N = 381)	Brandenburg (N = 45)	Infantis (N = 93)	Virchow (N = 55)	Hadar (N = 20)	Java (N = 128)	Dublin (N = 14)
Amoxicillin	39,1	86,0	35,0	2,9	0	11,8	12,7	35,0	58,6	0
Cefotaxime	0,9	0	5,0	0	0	1,1	1,8	0	2,3	0
Cefuroxime	1,1	0	5	0,3	0	1,1	1,8	5,0	4,7	0
Imipenem	0	0	0	0	0	0	0	0	0	0
Gentamicin	0,7	0	5,0	0,3	2,2	1,1	0	0	1,6	0
Neomycin	0,2	0,8	0	0	0	1,1	0	0	0	0
Doxycycline	42,4	57,4	51,7	1,6	13,3	9,7	9,1	85,0	15,6	0
Trim/sulpha	12,6	11,6	31,7	0,8	11,1	3,2	5,5	0	88,3	0
Trimethoprim	12,6	11,6	31,7	0,8	11,1	3,2	5,5	0	96,9	0
Ciprofloxacin	0	0	0	0	0	0	0	0	0	0
Flumequine	2,3	3,9	6,7	6,0	0	2,2	85,5	70,0	35,9	7,1
Chloramphenicol	26,2	76,0	10,0	0	0	3,2	0	0	0,8	14,3
Florfenicol	19,2	63,6	1,7	0	0	1,1	0	0	0,8	0

Table 4. Resistance percentages of *S.* Enteritidis, *S.* Typhimurium and *S.* Paratyphi B var. Java from different sources in 2002.

	S. Typhimurium				*S.* Enteritidis		*S.* Paratyphi B var. Java	
	Human* (N = 259)	Poultry* (N = 27)	Pigs* (N = 89)	Cattle* (N = 22)	Human (N = 319)	Poultry (N = 36)	Human (N = 4)	Poultry (N = 117)
Amoxicillin	41,1	48,1	30,3	59,1	2,5	8,3	50	59,8
Cefotaxime	0,78	3,7	0	0	0	0	0	1,7
Cefuroxime	0,78	7,4	0	0	0,3	0	0	4,3
Imipenem	0	0	0	0	0	0	0	0
Gentamicin	1,2	0	0	0	0,3	0	0	0,9
Neomycin	0,4	0	0	0	0	0	0	0
Doxycycline	42,7	22,2	51,7	59,1	1,9	0	25	16,2
Trim/sulpha	13,6	7,4	13,5	22,8	0,6	2,8	25	90,6
Trimethoprim	13,6	7,4	13,5	22,8	0,6	2,8	25	99,1
Ciprofloxacin	0	0	0	0	0	0	0	0
Flumequine	2,3	0	3,4	0	6,6	2,8	25	36,8
Chloramphenicol	28,3	14,8	20,2	50	0	0	25	0
Florfenicol	19,8	7,4	13,5	45,5	0	0	25	0

*: 30,2%, 29,6%, 19,1%, and 54,5% DT104 respectively

In Table 3 resistance percentages are presented for the most prevalent serotypes isolated in The Netherlands in 2002, including DT104 and ft507. In Table 4 resistance percentages for *S.* Typhimurium, *S.* Enteritidis and *S.* Java are presented for humans and the most important food-animal species. Resistance percentages of *S.* Typhimurium in 2002 were strongly determined by the relatively large numbers of DT104 (29%) and ft 507 (13,5%) included. These phage types were the predominant phage types of *S.* Typhimurium isolated in 2002, both in food-animals and in humans. In 2002 ten flumequine resistant *S.* Typhimurium isolates were found, five were DT104, four ft 507 and one ft 61. Six of these strains were isolated from human patients, four from pigs and one (ft 61) from a quail. In 1999 three flumequine resistant DT104 were isolated, in 2000 one, in 2001 none and in 2002 five. All flumequine resistant isolates demonstrated reduced susceptibility to ciprofloxacin. Trends in resistance in *S.* Typhimurium are difficult to determine in all sources (Fig. 1) because of the

influence of the presence of multiple resistant clones. Specifically when the total numbers of strains per year are relatively small the variability in the resistance percentages is high (eg. in cattle).
In general *S.* Enteritidis was susceptible to most antibiotics tested. However resistance to flumequine was relatively high and similar to that found in 2001 (8%). The twenty-two flumequine resistant strains were all but one isolated from human patients. Like in 2001 68% of these strains were *S.* Enteritidis pt1 and 22% pt4. In The Netherlands in 2002 the predominant phagetypes of *S.* Enteritidis isolated from human patients were pt4 (51,3%), pt21 (17,0%) and pt1 (9,2%). In strains isolated from poultry the predominant phagetypes were pt4 (49,6%), pt21 (12,9%), pt7 (10,1%) and pt1 (9,4%). Although in The Netherlands human salmonellosis (non-typhoid) is considered to be a domestically acquired disease, at least 18% of the human infections with pt1 in 2002 were travel related (Report on trends and sources of zoonotic agents in the EU, 2002, The Netherlands). Moreover the flumequine resistance data from this monitoring programme indicate that the majority of the flumequine resistant *S.* Enteritidis isolates did not originate from Dutch poultry, but from other sources like imported poultry products. This is reflected by the resistance patterns of human and poultry isolates of *S.* Enteritidis (Fig. 2). Also in the UK in the last two years a rapid increase was observed in quinolone resistance in *S.* Enteritidis pt1. This increase was related to imported eggs from Spain (personal communication, dr. J. Threlfall, Public Health Laboratory Services, Collindale, UK). Moreover, in 2002, in broilers as well as in poultry meat at retail, *S.* Enteritidis was found sporadically (1,9% of all isolates). Only in human isolates resistance to aminoglycosides, cefalosporins and fenicols was detected, again indicating that other sources than Dutch poultry exist.
S. Java was the predominant serotype isolated from broilers. Clonal distribution in The Netherlands and Germany in broiler production of a strain 100% resistant to trimethoprim and furazolidon and increasingly resistant to flumequine is the cause. In 2002 only 4 strains were isolated from human patients and only one of these strains demonstrated the phenotype typical for the clone (TmpFlu resistant). From poultry 117 strains were isolated of which 116 had the phenotype typical for the clone. Flumequine resistance in *S.* Java isolated from poultry has increased from 23% in 2001 to 36.8% in 2002 (fig. 3). No strains were found with ciprofloxacin MICs > 2 µg/ml.

Figure 1. Trends in resistance percentages of *S.* Typhimurium isolated from humans and food-animals from 1999 - 2002

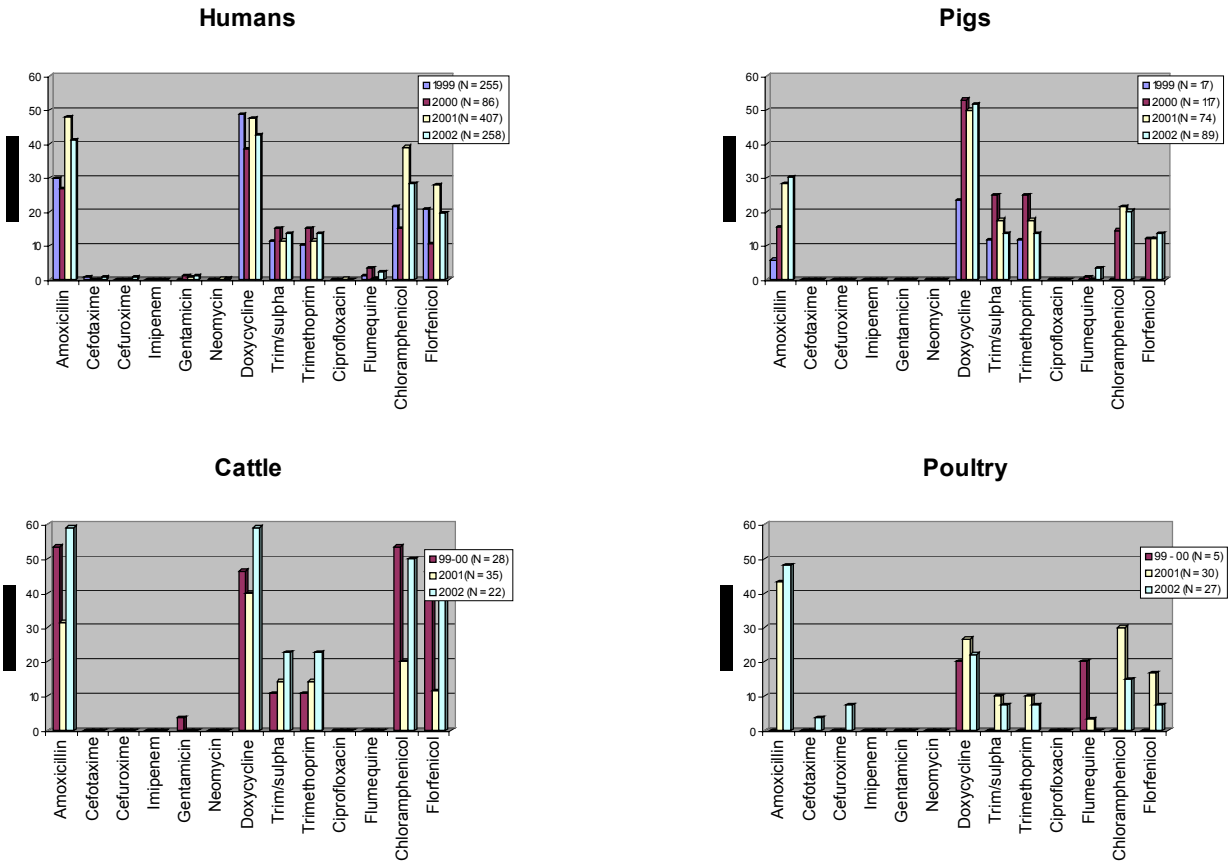


Figure 2. Trends in resistance percentages of *S.* Enteritidis isolated from humans and poultry (predominantly from layers, reproduction animals and poultry meat of undefined origin) from 1999 - 2002.

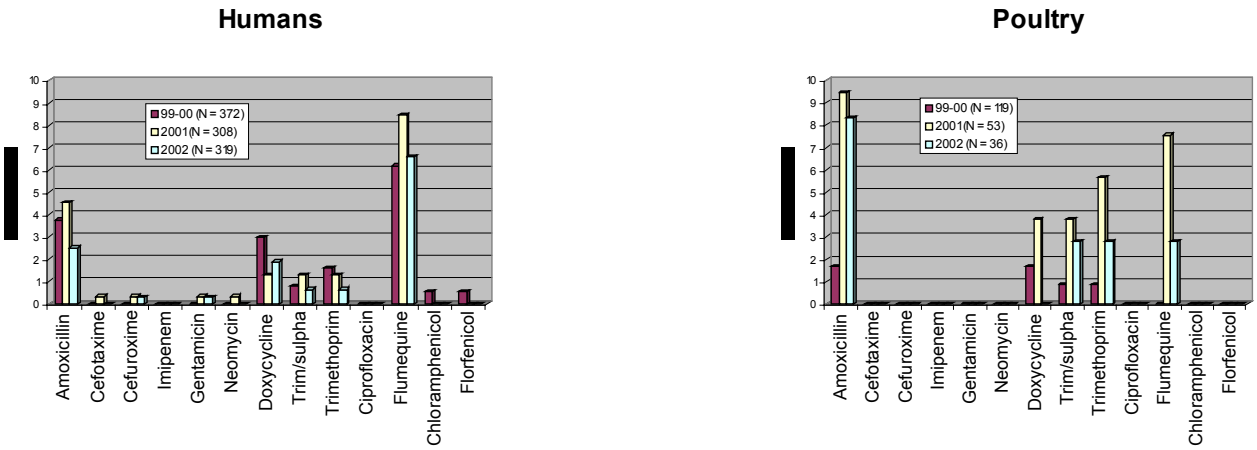
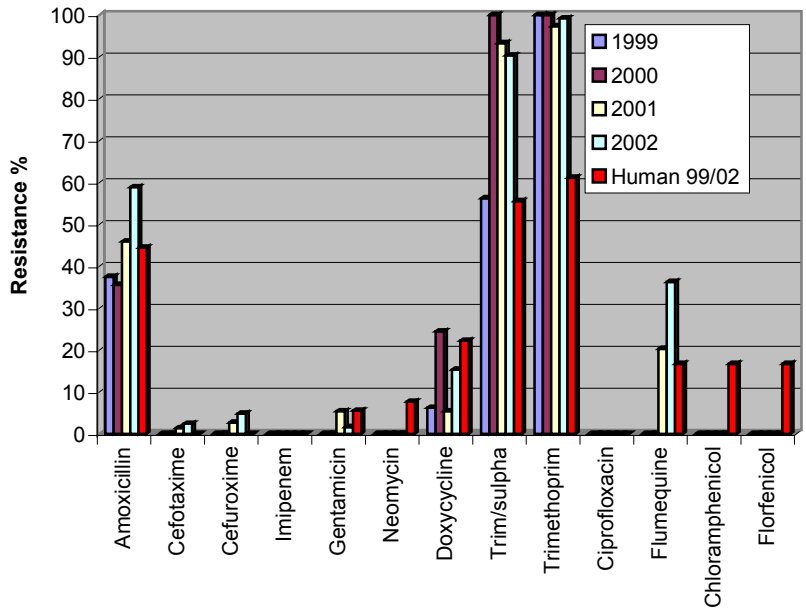


Figure 3. Trends in resistance percentages of *S.* Paratyphi B var. Java isolated from poultry from 1999 – 2002 and humans (red bars indicate all humans isolates from 1999 – 2002 (N = 18))

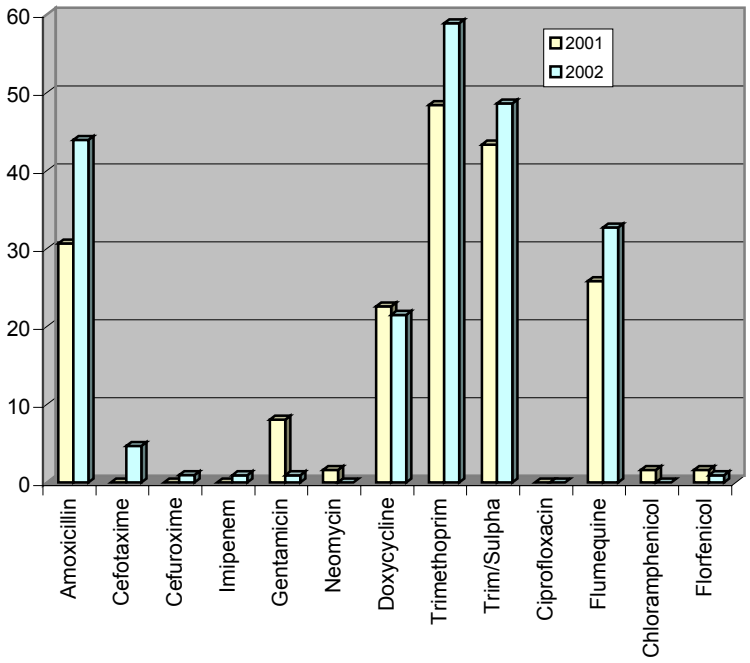


Salmonella spp. in raw meat products of food-animals

Tabel 5. Resistance % of *Salmonella* spp. isolated from chicken-, cattle- and pig products in 2002

	Chicken N = 107	Cattle N = 10	Pigs N = 8
Amoxicilline	43.9	10	0
Cefotaxime	4.7	0	0
Cefuroxime	1.0	0	12.5
Imipenem	0.9	0	0
Gentamicin	0.9	0	0
Neomycin	0.0	0	0
Doxycycline	21.5	10	25
Trim/suplha	48.6	20	37.5
Trimethoprim	58.9	20	37.5
Ciprofloxacin	0.0	0	0
Flumequine	32.7	0	0
Chloramphenicol	0.0	0	0
Florfenicol	0.9	0	0

Figure 4. Trends in resistance % of *Salmonella* spp. isolated from chicken products in the Netherlands in 2001 (N = 62) and 2002 (N = 107)



Chicken products were sampled in a statistically representative way so that contamination fractions and serotype distribution are representative for poultry meat at retail for the whole of The Netherlands (see chapter V appendices). The observed resistance patterns and trends in the chicken isolates are strongly determined by the large contribution of *S.* Java (53.5% of all *Salmonella* isolates from poultry products in 2002 and 43,2% in 2001, see appendices Table 5). The resistance percentages show a general tendency to increase. Cefotaxime resistance is

present at a level similar as found in *S.* Typhimurium and *S.* Java, isolated from poultry (Table 1, Figs. 1 and 3).

Salmonella spp. in animal feeds

In Table 6 resistance data are presented for salmonella's isolated from animal feeds. The resistance percentages were much lower than those for the human and food-animal isolates. In 2001 and 2002 a large variety of in general uncommon serotypes in humans and farm animals were isolated. Only 9 *S.* Typhimurium strains were isolated and tested from fodder, of which 3 were DT104. *S.* Enteritidis and *S.* Java were rarely isolated from animal feeds (15 and 2 out of 1257 isolates, respectively) and were not tested for susceptibility. Although animal feeds may be a source for introduction of antibiotic resistant *Salmonella* spp. in food animals, the contribution to the resistance levels in the predominant serotypes seems to be small.

Table 6. Numbers of isolates of *Salmonella* spp. per single and or compound feed type tested for susceptibility and resistance percentages (R%), 2001 and 2002 combined.

Feed source (N)	Fishmeal (29)	Animalmeal (6)	Fodder,single (76)	Fodder,composite (3)	Soy.beans,toasted (33)	Soy bean.expeller/extracted/hulls (27)	Canola/rapeseed expeller/extracted (71)	Cattle.feed.meal/piece (4)	Pig.feed(piece/meal (1)	Chicken.feed (layers).meal/piece (10)	Chicken.feed (broilers).meal/piece (3)	Dog.food(meal/piece (3)	Other.feed(meal/piece (29)	R%
Amoxicilline	1	-	3	-	-	-	1	-	-	-	-	-	1	2
Cefotaxime	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Cefuroxime	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Imipenem	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Gentamicin	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Neomycin	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Doxycycline	-	-	4	-	1	2	1	-	-	-	-	-	2	3
Trim/sulpha	1	-	2	-	-	1	-	-	-	-	-	-	-	1
Trimethoprim	1	-	2	-	-	1	-	-	-	-	-	-	-	1
Ciprofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Flumequine	-	-	2	-	-	-	-	-	-	-	-	-	2	1
Chloramphenicol	-	-	3	-	-	-	1	-	-	-	-	-	1	2
Florfenicol	-	-	1	-	-	-	1	-	-	-	-	-	1	1

Campylobacter spp.

Table 7. MIC distribution (in %) for all campylobacters (N =116) isolated from slaughter pigs and broilers in The Netherlands in 2002

	MIC distribution (µg/ml)														
Total 2002	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**R%**
Amoxicillin	-	-	3,4	9,2	26,1	24,4	19,3	0,8	3,4	13,4	-	-	-	-	16,8
Gentamicin	-	61,3	37,8	0,8	-	-	-	-	-	-	-	-	-	-	0,0
Neomycin	-	-	37,0	50,4	9,2	-	0,8	-	2,5	-	-	-	-	-	2,5
Streptomycin	-	-	-	42,0	5,0	-	1,7	3,4	18,5	13,4	0,8	15,1	-	-	51,3
Doxycycline	23,5	7,6	8,4	0,8	0,8	1,7	16,8	26,9	13,4	-	-	-	-	-	57,1
Trim/sulpha	-	1,7	7,6	12,6	18,5	19,3	3,4	16,8	18,5	1,7	-	-	-	-	37,0
Sulphamethoxazole	-	-	-	-	-	-	7,6	8,4	17,6	20,2	10,1	9,2	25,2	1,7	26,9
Ciprofloxacin	36,1	28,6	7,6	0,8	-	1,7	9,2	6,7	9,2	-	-	-	-	-	26,9
Nalidixic acid	-	-	-	0,8	12,6	26,9	28,6	5,0	-	1,7	15,1	9,2	-	-	26,1
Erythromycin	-	-	-	10,9	32,8	21,8	17,6	5,9	5,0	1,7	4,2	-	-	-	34,5
Metronidazole	-	-	5,0	16,0	19,3	10,1	5,9	5,9	15,1	15,1	7,6	-	-	-	37,8
Chloramphenicol	-	-	-	-	26,1	36,1	31,1	6,7	-	-	-	-	-	-	0,0

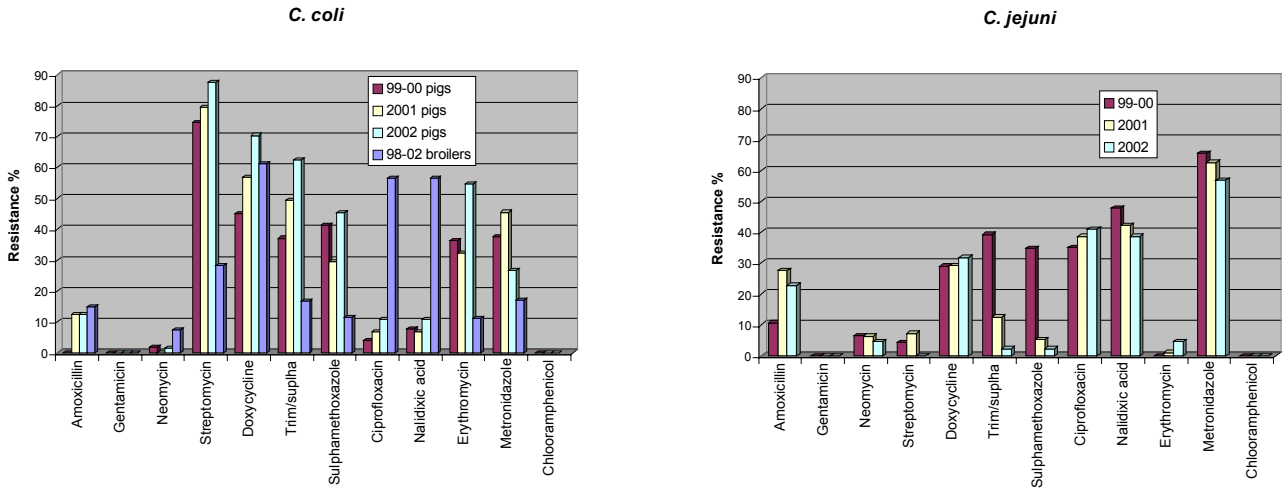
The white areas indicate the dilution range tested for each antimicrobial agent. Values above this range indicate MIC values > the highest concentration in the range. Values at the lowest concentration tested indicate MIC-values ≤ the lowest concentration in the range. Vertical bars indicate the breakpoints.

Table 7 presents the MIC-distributions and resistance percentages for all campylobacters isolated from broilers and slaughter pigs in 2002. In Table 8 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.
Substantial resistance percentages can be observed for amoxicillin, nalidixic acid, ciprofloxacin, doxycycline, erythromycin, metronidazole, (potentiated) sulphonamides and streptomycin. However differences in level of resistance exist both between *C. jejuni* and *C. coli*, and between pigs and broilers. *C. coli* isolated from broilers is more resistant to antibiotics than *C. jejuni* (Table 8 , Fig. 5). Amongst others, the resistance percentages for the quinolones (ciprofloxacin and nalidixic acid) and erythromycin are much higher in *C. coli.*

Table 8. Resistance percentages of *C. jejuni* and *C. coli* isolated from broilers and slaughter pigs in 2002

Campylobacter spp.	Broilers		Pigs
2002	*C. jejuni* (N = 44)	*C. coli* (N = 10)	*C. coli* (N = 64)
Amoxicillin	22,7	20,0	12,5
Gentamicin	0,0	0,0	0,0
Neomycin	4,5	0,0	1,6
Streptomycin	0,0	50,0	87,5
Doxycycline	31,8	90,0	70,3
Trim/sulpha	2,3	30,0	62,5
Sulphamethoxazole	2,3	20,0	45,3
Ciprofloxacin	40,9	70,0	10,9
Nalidixic acid	38,6	70,0	10,9
Erythromycin	4,5	40,0	54,7
Metronidazole	56,8	30,0	26,6
Chloramphenicol	0,0	0,0	0,0

Figure 5. Trends in resistance percentages of *C. coli* isolated from slaughter pigs and broilers, and *C. jejuni* isolated from broilers from 1999 - 2002



In pigs resistance to trimethoprim/sulphamethoxazole, doxycycline, streptomycin and erythromycin showed a tendency to increase. In poultry no real trends could be observed. In 2002 surprisingly, one *C. jejuni* strain was isolated from broilers resistant to ciprofloxacin (MIC ≥ 16 µg/ml) and susceptible to nalidixic acid (MIC 8 µg/ml), which was confirmed by Etest.

Figure 6. Trends in resistance % of *Campylobacter* spp. isolated from humans isolated between 1993 and 2002 (400 – 700 isolates/year) at the regional Publuc Health Laboratories (PHLs) of Arnhem and Heerlen covering 990.000 inhabitants from 1993 - 2002

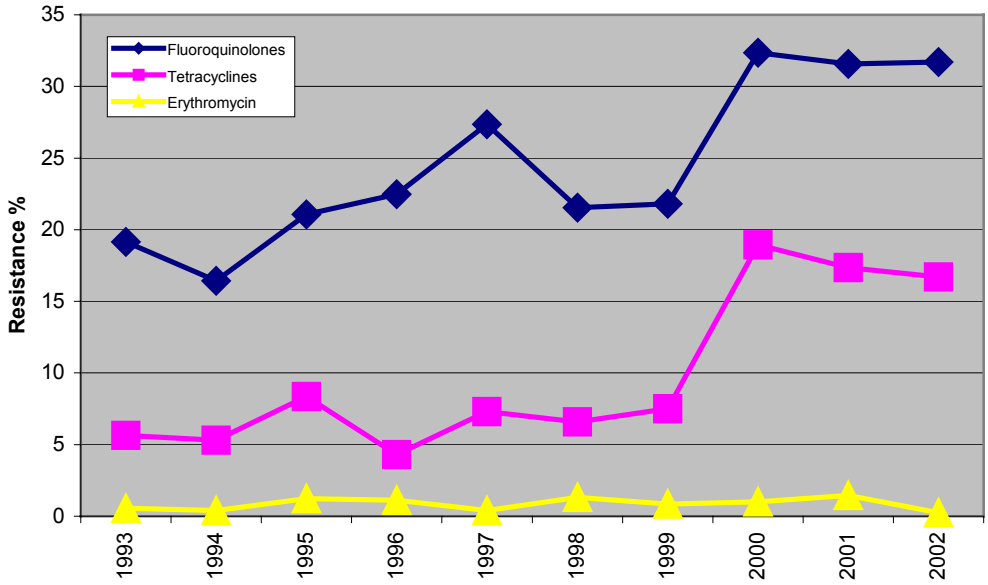


Figure 6 shows that in human Campylobacter spp. resistance to fluoroquinolones (data are based on disk diffusion tests for norfloxacin, ofloxacin and ciprofloxacin) slowly increased in the last decade. In 2000 both resistance to fluoroquinolones and tetracyclines increased suddenly approximately 10%. A biological explanation for this phenomenon does not exist. Resistance to macrolides remained stable at a very low level. Because at the PHLs the *Campylobacter* spp. were not typed to the species level, differences between C. jejuni and C. coli can only be speculated upon.

Table 9. Domestically acquired and travel related strains of *C. jejuni* and *C. coli* isolated from humans in 2002 from all 16 Dutch PHLs covering > 60% of the Dutch population

Casa-project 2002 - 2003	Domestically acquired				Travel related			
	C. jejuni		C. coli		C. jejuni		C. coli	
	N	R%	N	R%	N	R%	N	R%
Fluoroquinolone	2397	30,9	176	39,2	280	52,5	44	59,1
Tetracycline	1870	17,9	153	35,3	236	28,8	35	34,3
Erythromycin	2297	3,9	175	6,3	262	2,7	38	10,5

The 2002 resistance percentages presented in Figure 6 are confirmed by disk diffusion data from all regional PHLs covering over 60% of all 16.2 million Dutch inhabitants (CaSa-project) (Table 9). The species identification of all isolates was confirmed by PCR at Animal Sciences Group in Lelystad, The Netherlands. The resistance percentages of domestically acquired *C. jejuni* for fluoroquinolones and tetracyclines were similar as the PHLs data and those found in C. *jejuni* isolates from poultry. Erythromycin resistance was not detected in

poultry *C. jejuni* isolates until 2002. The observed resistance to erythromycin may indicate that for human infections, other sources than Dutch poultry exist. However it may also indicate methodological problems with the disk diffusion test and breakpoint used for erythromycin. As expected, resistance levels in *C. coli* were higher than in *C. jejuni.* Resistance fluoroquinolones was clearly higher in travel related infection than in those domestically acquired.

Shigella toxin producing *E. coli* O157

Table 10. Sources for *E. coli* O157 isolated in The Netherlands in 2002.

Sources in 2002	N
Human	45
Cattle	49
Veal calf	33
Water	12
Chicken	1
Other	7
Total N	147

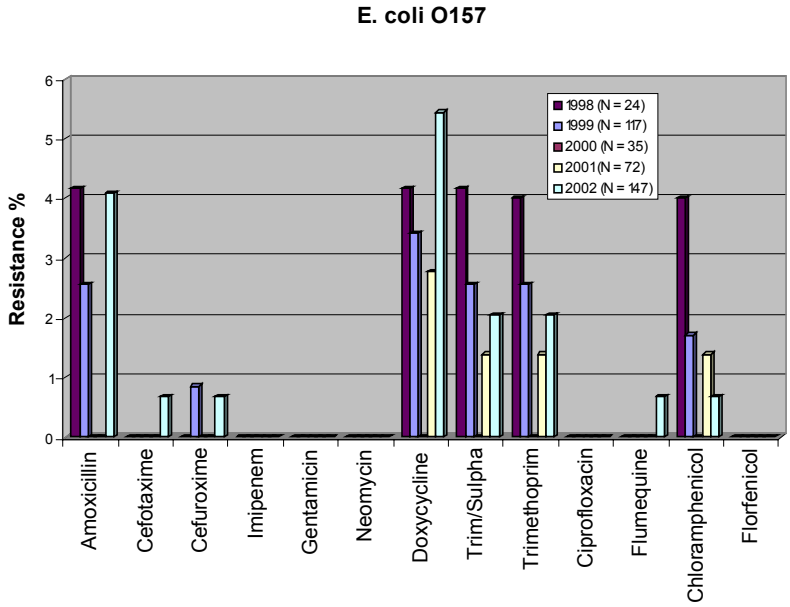
In 2002 147 strains of *E. coli* O157 were sent to RIVM for typing purposes or isolated from specimens taken from food-animals or water in an attempt to trace a human clinical infection (Table 10). Table 11 demonstrates that in general the resistance levels are low. One isolate was resistant to cefotaxime and cefuroxime and therefore suspected to be positive for Extended Spectrum Beta-Lactamases (ESBL's). This was confirmed by Etest (MIC ceftazidime/ceftazidime-clavulanic acid > 32 µg/ml and 3 µg/ml, respectively).

Table 11. MIC distribution (in %) for *E. coli* O157 (N = 147) isolated in The Netherlands in 2002 from different sources

	MIC distribution (µg/ml)															
E. coli* O157 *2002	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R%**
Amoxicillin	-	-	-	-	-	-	-	3,4	89,1	3,4	-	-	4,1	-	-	4,1
Cefotaxime	-	-	-	98,6	0,7	-	-	-	-	-	0,7	-	-	-	-	0,7
Cefuroxime	-	-	-	-	-	-		-	-	-	99,3	-	-	0,7	-	0,7
Imipenem	-	-	-	91,2	8,2	0,7	-	-	-	-	-	-	-	-	-	0,0
Gentamicin	-	-	-	-	62,6	35,4	2,0	-	-	-	-	-	-	-	-	0,0
Neomycin	-	-	-	-	-	-	91,8	8,2	-	-	-	-	-	-	-	0,0
Doxycycline	-	-	-	-	-	-	4,1	88,4	2,0	1,4	1,4	2,7	-	-	-	5,4
Trim/Sulpha	-	-	-	-	98,0	-	-	-	-	-	-	-	2,0	-	-	2,0
Trimethoprim	-	-	-	-	-	98,0	-	-	-	-	-	-	-	2,0	-	2,0
Ciprofloxacin	-	-	99,3	0,7	-	-	-	-	-	-	-	-	-	-	-	0,0
Flumequin	-	-	-	-	-	21,1	72,8	5,4	-	0,7	-	-	-	-	-	0,7
Chloramphenicol	-	-	-	-	-	-	-	-	11,6	87,1	0,7	-	-	-	1,0	1,0
Florfenicol	-	-	-	-	-	-	-	1,4	29,9	68,0	0,7	-	-	-	-	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 7. Trends in resistance percentages of *E. coli* O157 isolated in The Netherlands from 1998 - 2002



From 1998 until 2002, 396 strains of *E. coli* O157 were isolated in The Netherlands and tested for susceptibility. The resistance percentages were less than 6% for all antibiotics tested throughout the years, and trends could not be detected (Fig. 7). Of these strains 378 (95,4%) were susceptible to all antibiotics tested. Seven strains were resistant to one antibiotic (5 doxycycline, 1 flumequine, and 1 amoxicillin), four strains were resistant to two antibiotics (1 AmoxicillinTrimethoprim, 2 AmoxicillinDoxycycline, 1 DoxycyclineTrimethoprim), three strains were resistant to three antibiotics (2 AmoxicillinDoxycyclineTrimethoprim, 1 DoxycyclineTrimethoprimChloramphenicol) and four strains were resistant to four antibiotics (3 AmoxicillinDoxycyclineTrimethoprimChloramphenicol, 1 CefotaximeDoxycycline-TrimethoprimChloramphenicol (ESBL-positive)). All trimethoprim and trimethoprim-sulphamethoxazole resistant isolates were examined with PCR for the presence of class I integrons as genetic basis for multiple resistance. Six of the multiple-resistant isolates (4 human, 2 non-human) were positive for class I integrons. These strains harboured a 1550 bp large fragment in the conserved segment (CS) of the integron that contains trimethoprim and aminoglycoside (streptomycin and spectinomycin) resistance genes (dfr1a/aadA1a). Class I integrons with identical CS-fragments were detected in multiple resistant commensal *E. coli* strains isolated from veal calves, pigs, broilers, meat products and multiple resistant ESBL-positive *Salmonella* spp. isolated from human clinical submissions, and poultry. This indicates that horizontal transmission of class I integrons occurs between commensal *E. coli*, *E. coli* O157 and *Salmonella* spp.

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 12 and 13 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 calsses of antibiotics, amoxicillin, doxycycline, (potentiated) trimethoprim and chloramphenicol showed the highest resistance levels (Table 12). Moreover, the resistance levels show a tendency to increase (Fig. 8). The increase observed for the older classes of antibiotics 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.

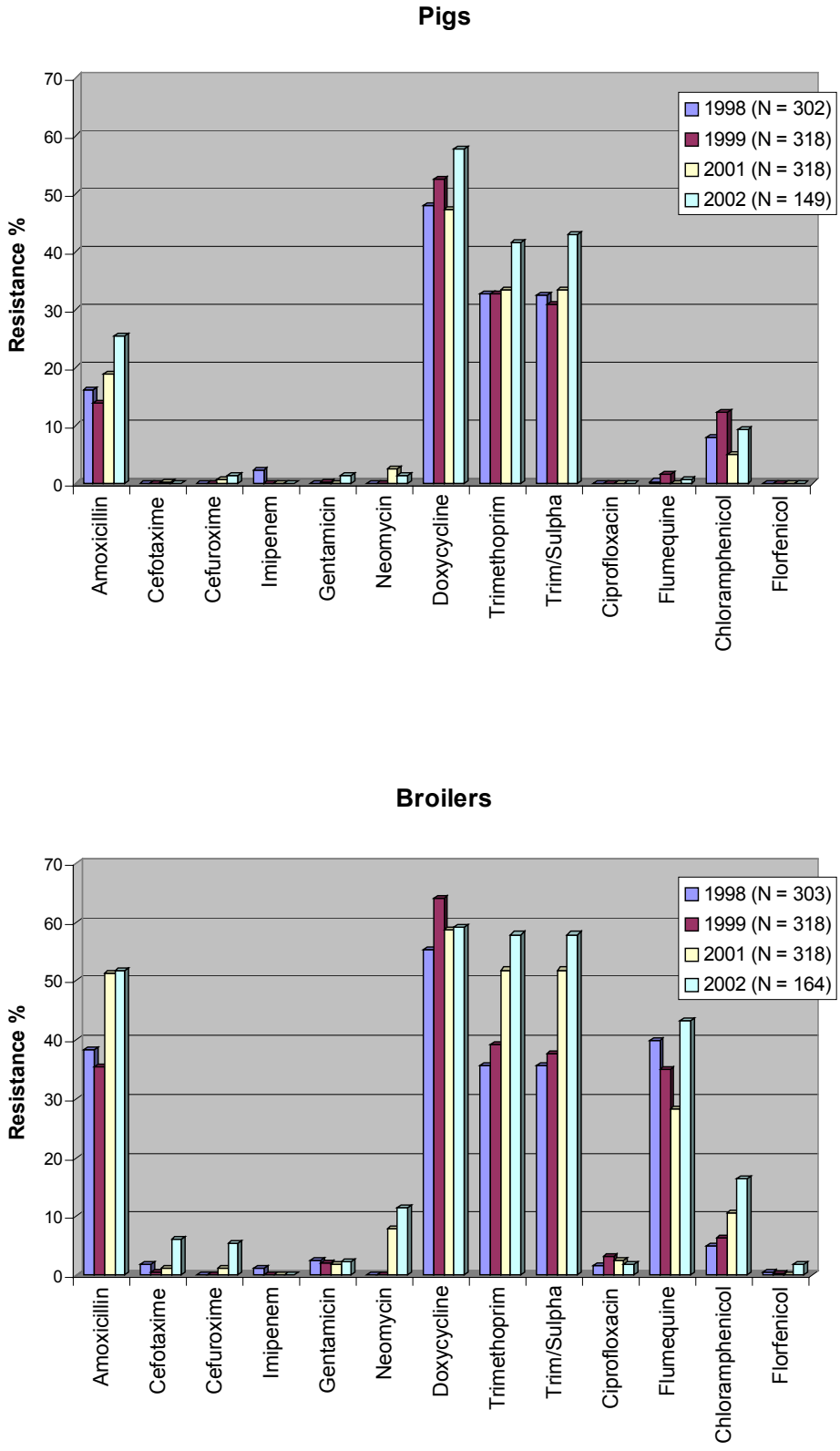
In broilers resistance to flumequine was very substantial (43,3%). Following the observed decrease in flumequine resistance in 2001, the level is again similar as found in 1998. Whether this is due to increased usage or just normal variation is not clear. In broilers ten cefotaxime resistant strains were isolated that were suspected to be ESBL-positive. Since cefalosporins are not used in broiler production, co-selection by the use of another beta-lactam antibiotic or an antibiotic of another class may have been the cause.

Table 12. MIC distributions (in %) for *E. coli* isolated from slaughter pigs (N = 149) and broilers (N = 164) in The Netherlands in 2002.

2002	**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	-	-	-	-	-	-	2,0	26,2	39,6	6,7	-	0,7	24,8	-	-	-	25,5
Cefotaxime	-	-	-	96,6	2,0	0,7	0,7	-	-	-	-	-	-	-	-	-	0,0
Cefuroxime	-	-	-	-	-	-	-	-	-	-	98,7	1,3	-	-	-	-	1,3
Imipenem	-	-	-	89,9	10,1	-	-	-	-	-	-	-	-	-	-	-	0,0
Gentamicin	-	-	-	-	14,8	59,7	17,4	5,4	1,3	-	-	0,7	0,7	-	-	-	1,3
Neomycin	-	-	-	-	-	-	79,9	15,4	3,4	-	-	1,3	-	-	-	-	1,3
Doxycycline	-	-	-	-	-	0,7	8,1	22,1	11,4	18,1	25,5	13,4	0,7	-	-	-	57,7
Trim/Sulpha	-	-	-	-	49,7	6,0	2,0	0,7	0,7	-	-	-	40,9	-	-	-	41,6
Trimethoprim	-	-	-	-	-	44,3	9,4	2,7	0,7	-	-	-	-	43,0	-	-	43,0
Ciprofloxacin	-	-	99,3	-	-	-	0,7	-	-	-	-	-	-	-	-	-	0,0
Flumequine	-	-	-	-	-	69,8	29,5	-	-	-	-	-	1,0	-	-	-	1,0
Chloramphenicol	-	-	-	-	-	-	-	-	10,1	70,5	10,1	5,4	1,3	-	2,7	-	9,4
Florfenicol	-	-	-	-	-	-	-	0,7	31,5	61,7	6,0	-	-	-	-	-	0,0
	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	-	-	-	-	-	-	1,8	14,0	27,4	4,9	-	-	51,8	-	-	-	51,8
Cefotaxime	-	-	-	91,5	1,2	0,6	0,6	1,2	1,2	1,2	2,4	-	-	-	-	-	6,1
Cefuroxime	-	-	-	-	-	-	-	-	-	-	94,5	1,8	1,2	2,4	-	-	5,5
Imipenem	-	-	-	81,7	18,3	-	-	-	-	-	-	-	-	-	-	-	0,0
Gentamicin	-	-	-	-	10,4	62,8	19,5	4,3	-	0,6	0,6	1,8	-	-	-	-	2,4
Neomycin	-	-	-	-	-	-	69,5	14,6	4,3	-	-	4,9	4,9	0,6	1,2	-	11,6
Doxycycline	-	-	-	-	-	2,4	5,5	21,3	11,6	16,5	28,0	9,1	5,5	-	-	-	59,1
Trim/Sulpha	-	-	-	-	34,1	6,7	0,6	0,6	-	-	-	-	57,9	-	-	-	57,9
Trimethoprim	-	-	-	-	-	32,9	7,9	0,6	0,6	-	-	-	-	57,9	-	-	57,9
Ciprofloxacin	-	-	54,9	11,0	24,4	5,5	1,8	0,6	0,6	0,6	0,6	-	-	-	-	-	1,8
Flumequine	-	-	-	-	-	37,2	15,2	2,4	1,8	19,5	16,5	3,0	4,3	-	-	-	43,3
Chloramphenicol	-	-	-	-	-	-	-	-	5,5	67,1	11,0	1,2	1,2	2,4	11,6	-	16,5
Florfenicol	-	-	-	-	-	-	-	1,8	31,7	55,5	9,1	1,2	0,6	-	-	-	1,8

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

Figure 8. Trends in resistance percentages of *E. coli* isolated from slaughter pigs and broilers in The Netherlands from 1998 - 2002



E. coli in raw meat products of food-animals

Table 13. Resistance % of *E. coli* isolated from raw meat products of poultry, adult cattle, calves and pigs in The Netherlands in 2002

	Poultry N = 120	Adult cattle N = 97	Calves N = 18	Pigs N = 54
Amoxicillin	45,0	7,2	16,7	13,2
Cefotaxime	2,5	0,0	0,0	1,9
Cefuroxime	1,7	0,0	0,0	3,7
Imipenem	0,0	0,0	0,0	3,7
Gentamicin	0,0	0,0	0,0	0,0
Neomycin	6,7	4,1	0,0	0,0
Doxycycline	45,8	11,3	50,0	18,5
Trim/Sulpha	36,7	4,1	44,4	13,0
Trimethoprim	38,3	5,2	44,4	16,7
Ciprofloxacin	4,2	0,0	0,0	0,0
Flumequine	19,2	1,0	0,0	0,0
Chloramphenicol	10,8	5,2	22,2	0,0
Florfenicol	0,8	0,0	0,0	0,0

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*. In *E. coli* from raw meat products of adult cattle, resistance percentages are much lower than in poultry and pigs. In the strains from calves resistance levels are similar as or higher than those from poultry and pigs. In strains isolated from pig products, resistance percentages were lower than those found at slaughter. Moreover in pig products cefotaxime and imipenem resistant strains were found, which were not found in strains isolated at slaughter.
The resistance patterns: amoxicillin/trimethoprim/sulphonamides/chloramphenicol, indicates the common presence of integrons, genetic structures involved in transmission of multiple resistance between enterobacteriacea of animal origin.

Enterococcus faecium, Enterococcus faecalis

In *E. faecium* isolated from broilers, next to doxycycline, the highest resistance percentages were found for those antibiotics representing the growth promoters: bacitracin, flavomycin (intrinsic resistance), avilamycin in broilers, erythromycin and tilmicosin (macrolides), and virginiamycin and quinupristin/dalfopristin (Synercid®) (streptogramins) (Table 14). Resistance to the glycopeptides vancomycin and teicoplanin remained stable, which may be due to co-selection by therapeutic use of macrolides. Amoxicillin and ciprofloxacin resistant strains were incidentally isolated from broilers. High-level streptomycin resistant strains were present in both animal species but in a higher percentage in broilers.

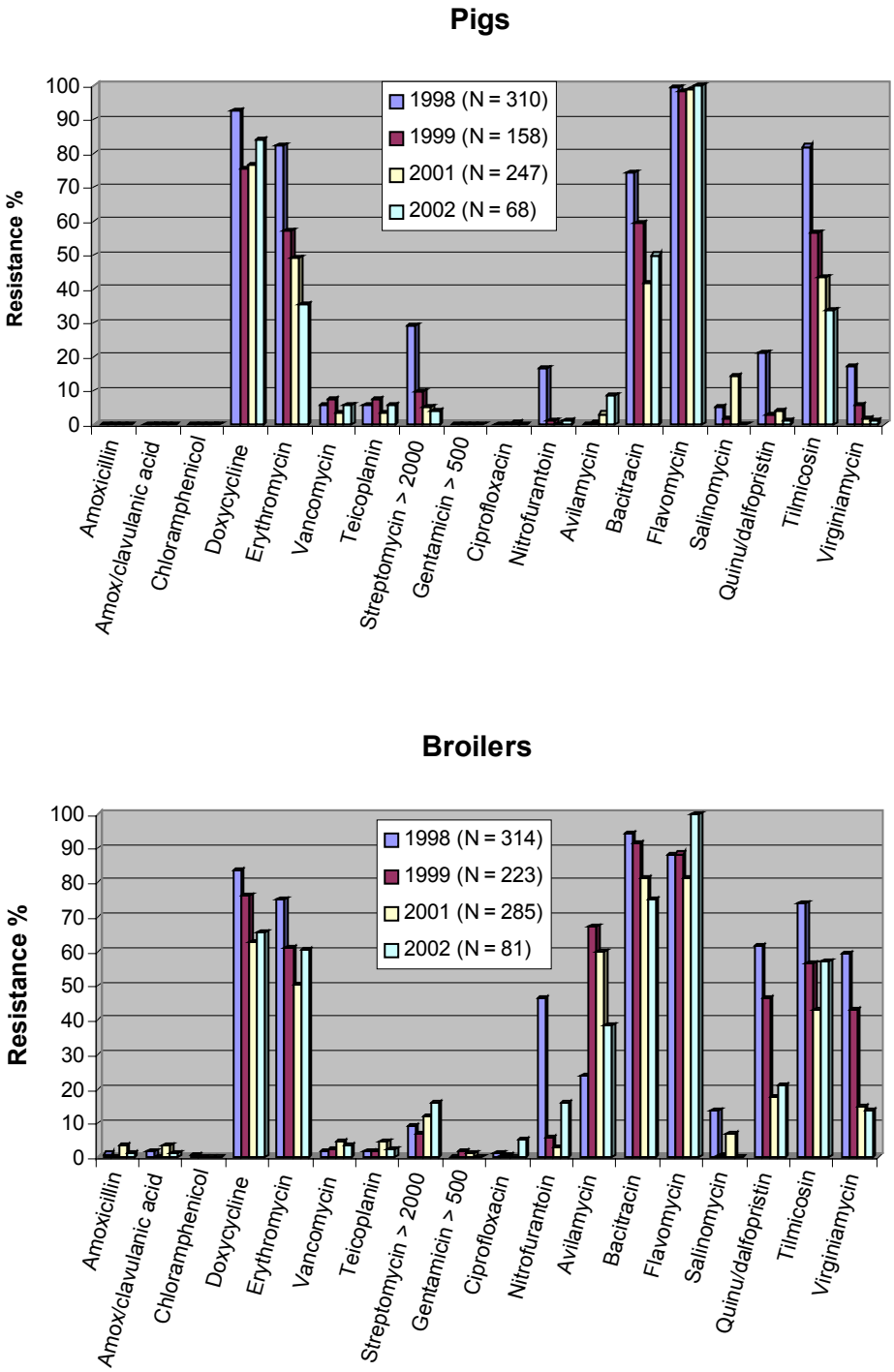
In slaughter pigs the tendency of resistance to decrease continued in 2002 for the macrolides, erythromycin and tilmicosin, the streptogramins, virginiamycin and quinupristin/dalfopristine and high-level streptomycin resistance (Fig. 9). Resistance to bacitracin shows a tendency to increase again after a decrease in the past years; resistance to doxycycline, avilamycin and salinomycin show a tendency to increase. For the latter three antibiotics an increase in usage may be the cause, but accurate data are lacking. In broilers the tendency until 2001 of resistance to macrolides, streptogramins, doxycycline, nitrofurantoin to decrease has stopped or has even changed in a slight increase of resistance levels. Resistance to bacitracin continues to decrease slowly and resistance to avilamycin that increased very significantly after the ban of virginiamycin, tylosine/spiramycin and bacitracin as growth promoters in 1999, shows a decrease in 2002. A biological explanation for the observed trends is difficult to provide because accurate antibiotic usage data are lacking. Data on other potential factors like clonal distribution of strains with a specific phenotype are essentially missing.

Table 14. MIC distributions (In %) for *E. faecium isolated* from slaughter pigs (N = 68) and broilers (N = 81) in The Netherlands in 2002.

2002	MIC distribution (µg/ml)																
Pigs	**0,03**	**0,06**	**0,12**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**R %**
Amoxicillin	-	-	1,5	-	8,8	20,6	32,4	27,9	8,8	-	-	-	-	-	-	-	0,0
Amox/clav. acid	-	1,5	-	-	5,9	22,1	36,8	25,0	8,8	-	-	-	-	-	-	-	0,0
Chloramphenicol	-	-	-	-	-	-	-	1,5	76,5	20,6	1,5	-	-	-	-	-	0,0
Doxycycline	-	-	7,4	8,8	-	-	-	-	4,4	54,4	23,5	1,5	-	-	-	-	83,8
Erythromycin	-	-	2,9	-	1,5	1,5	54,4	4,4	1,5	-	2,9	-	30,9	-	-	-	35,3
Vancomycin	-	-	-	-	80,9	11,8	1,5	-	-	-	-	-	-	5,9	-	-	5,9
Teicoplanin	-	-	1,5	55,9	23,5	13,2	-	-	-	-	-	5,9	-	-	-	-	5,9
Streptomycin > 2000	3 strains MIC > 2000																4,4
Gentamicin > 500	0 MIC > 500																0,0
Ciprofloxacin	-	-	-	1,5	26,5	39,7	19,1	10,3	2,9	-	-	-	-	-	-	-	0,0
Nitrofurantoin	-	-	-	-	-	-	-	-	-	-	-	7,4	91,2	1,5	-	-	1,5
Avilamycin	-	-	-	-	-	-	2,9	61,8	26,5	-	-	-	8,8	-	-	-	8,8
Bacitracin	-	-	-	-	-	-	-	5,9	26,5	5,9	1,5	-	10,3	50,0	-	-	50,0
Flavomycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100,0	100,0
Salinomycin	-	-	-	-	-	10,3	33,8	5,9	32,4	17,6	-	-	-	-	-	-	0,0
Quinu/dalfopristin	-	-	-	-	4,4	-	61,8	32,4	1,5	-	-	-	-	-	-	-	1,5
Tilmicosin	-	-	-	-	-	-	-	1,5	1,5	39,7	23,5	-	-	-	1,5	32,4	33,8
Virginiamycin	-	-	-	4,4	-	29,4	50,0	11,8	2,9	1,5	-	-	-	-	-	-	1,5
	MIC distribution (µg/ml)																
Broilers	**0,03**	**0,06**	**0,12**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**R %**
Amoxicillin	-	1,2	1,2	2,5	13,6	33,3	12,3	30,9	3,7	-	1,2	-	-	-	-	-	1,2
Amox/clav. acid	-	1,2	-	8,6	11,1	29,6	16,0	29,6	2,5	-	1,2	-	-	-	-	-	1,2
Chloramphenicol	-	-	-	-	-	-	1,2	4,9	74,1	11,1	8,6	-	-	-	-	-	0,0
Doxycycline	-	3,7	21,0	7,4	1,2	-	-	1,2	17,3	25,9	22,2	-	-	-	-	-	65,4
Erythromycin	-	4,9	8,6	3,7	-	7,4	13,6	1,2	-	-	1,2	-	59,3	-	-	-	60,5
Vancomycin	-	-	-	-	54,3	35,8	6,2	-	-	-	-	-	1,2	2,5	-	-	3,7
Teicoplanin	-	-	4,9	16,0	59,3	16,0	-	-	-	1,2	-	2,5	-	-	-	-	2,5
Streptomycin > 2000	13 strains MIC > 2000																16,0
Gentamicin > 500	0 MIC > 500																0,0
Ciprofloxacin	-	-	-	2,5	-	8,6	27,2	49,4	7,4	3,7	1,2	-	-	-	-	-	4,9
Nitrofurantoin	-	-	-	-	-	-	-	-	-	3,7	2,5	21,0	56,8	16,0	-	-	16,0
Avilamycin	-	-	-	-	-	3,7	4,9	14,8	32,1	6,2	1,2	3,7	33,3	-	-	-	38,3
Bacitracin	-	-	-	-	-	-	2,5	1,2	2,5	4,9	-	7,4	6,2	75,3	-	-	75,3
Flavomycin	-	-	-	-	-	-	-	-	-	-	-	-	1,2	2,5	4,9	91,4	100,0
Salinomycin	-	-	-	-	-	4,9	14,8	17,3	59,3	3,7	-	-	-	-	-	-	0,0
Quinu/dalfopristin	-	-	-	-	18,5	8,6	17,3	34,6	12,3	1,2	7,4	-	-	-	-	-	21,0
Tilmicosin	-	-	-	-	-	-	-	1,2	2,5	16,0	22,2	1,2	1,2	-	-	55,6	56,8
Virginiamycin	-	-	-	6,2	17,3	13,6	29,6	11,1	8,6	4,9	-	8,6	-	-	-	-	13,6

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. Vertical bars indicate the breakpoint.

Figure 9. Trends in resistance percentages of *E. faecium* isolated from slaughter pigs and broilers in The Netherlands from 1998 - 2002



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

Table 15. Resistance % of *E. faecalis* and *E. faecium* isolated from chicken-, cattle-, and pig-products in the Netherlands in 2002

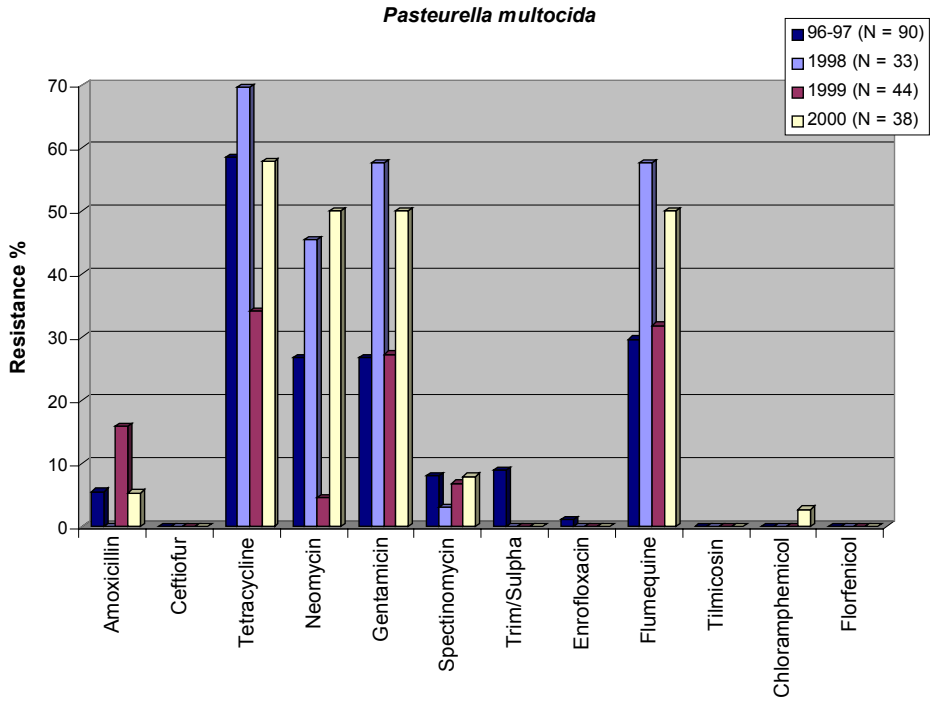
E. faecalis	Chicken N = 44	Cattle N = 65	Pigs N = 40
Amoxicillin	0.0	0.0	0.0
Doxycycline	47.7	26.2	25.0
Erythromycin	40.9	4.6	15.0
Vancomycin	0.0	0.0	0.0
Streptomycin > 1000 µg/ml	13.6	10.7	12.5
Streptomycin > 2000 µg/ml	9.1	3.1	2.5
Gentamicin	2.3	1.5	7.5
Ciprofloxacin	2.3	0.0	0.0
Bacitracin	47.7	1.5	15.0
Flavomycin	9.1	7.7	5.0
Salinomycin	0.0	1.5	7.5
Quinu/dalfopristin	11.4	6.2	15.0
E. faecium	Chicken N = 25	Cattle N = 43	Pigs N = 16
Amoxicillin	4.0	0.0	0.0
Doxycycline	44.0	2.3	18.8
Erythromycin	44.0	7.0	25.0
Vancomycin	0.0	0.0	0.0
Streptomycin > 1000 µg/ml	20	2.3	6.3
Streptomycin > 2000 µg/ml	8	0.0	0.0
Gentamicin	0.0	0.0	6.3
Ciprofloxacin	0.0	0.0	0.0
Bacitracin	56.0	21.4	6.3
Flavomycin	80.0	95.3	75.0
Salinomycin	0.0	0.0	6.3
Quinu/dalfopristin	4.0	0.0	0.0

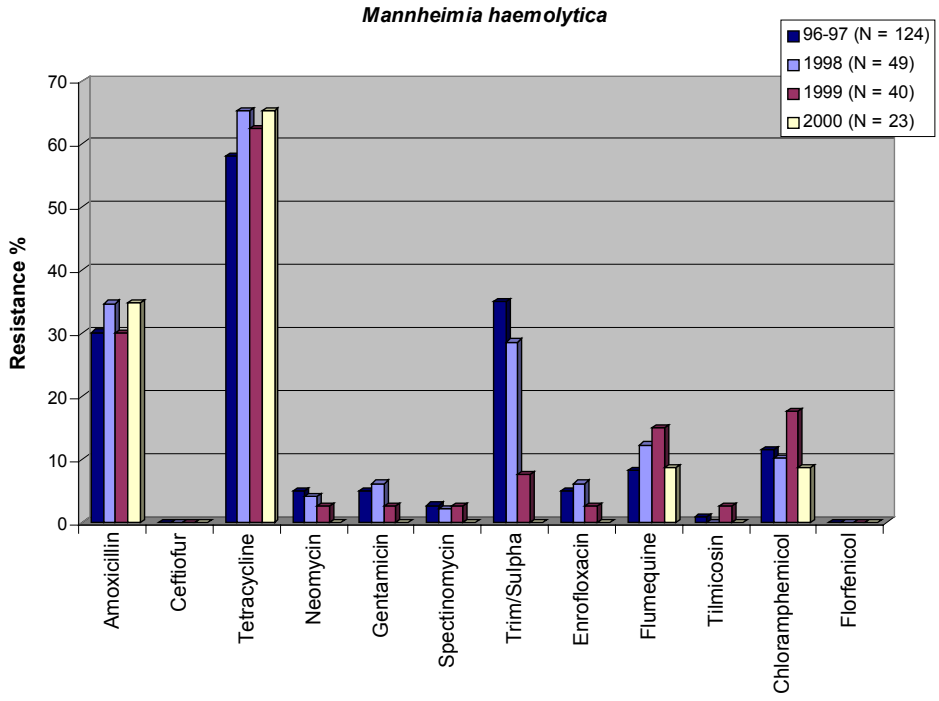
Resistance percentages in *E. faecium* isolated from raw meat products are lower than those found in isolates from food-animals. This may be selection bias due to the relatively small numbers tested. It may also indicate that subpopulations of strains adapted to survival in meat products exist. Vancomycin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

Animal pathogens

Respiratory tract pathogens: *Pasteurella multocida, Mannheimia haemolytica*

Figure 10. Trends in resistance percentages of *Pasteurella multocida* and *Mannheimia haemolytica* isolated from cattle in the Netherlands from 1996 - 2000





Since 1996, annually, approximately 100 *Pasteurella* multocida and *Mannheimia haemolytica* strains isolated and identified by the Animal Health Service in Deventer, The Netherlands, are sent to CIDC-Lelystad for quantitative susceptibility testing. In Fig. 10 the trends in resistance percentages are shown. 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. Differences in resistance percentages between both species exist for trimethoprim/sulpha, amoxicillin, enrofloxacin and chloramphenicol, which are higher in *M. haemolytica.* Resistance percentages of neomycin, gentamicin and flumequine are much higher in *P. multocida*.

Table 16. Resistance % of *Mannheimia haemolytica* (MHA) and *Pasteurella multocida* (PMU) isolated from veal calves and cattle in the Netherlands from 1996 - 2000

1996 – 2000	Veal calves		Other cattle	
	MHA	PMU	MHA	PMU
Number of strains	104	87	82	90
Amoxicillin	39,0	13,3	17,1	2,2
Ceftiofur	0	0	0	0
Tetracycline	74,3	63,2	41,5	25,8
Neomycin	4,8	35,7	2,4	16,9
Gentamicin	5,8	45,2	1,2	19,1
Spectinomycin	2,9	8,3	0	3,3
Trim/Sulpha	33,7	1,2	15,9	0
Enrofloxacin	5,8	1,1	2,4	0
Flumequine	14,4	50,0	6,1	20,0
Tilmicosin	1,0	0	1,2	0
Chloramphenicol	12,5	1,3	6,9	0
Florfenicol	0	0	0	0

In Table 16 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. For both bacterial species the resistance percentage are higher in strains from veal calves reflecting the higher selection pressure in these animals.

Enteric pathogens: *Brachyspira hyodysenteriae*

Tabel 17. MIC distribution for *B. hyodysenteriae* isolated from pigs in the Netherlands from 1998 - 2000

	0,015	0,03	0,06	0,125	0,25	0,5	1	2	4	8	16	64	128	256	512	N	R%
Tylosin	-	-	-	-	-	-	-	-	-	-	-	-	1	14	-	15	100
Tiamulin	-	-	-	15	-	-	-	-	-	-	-	-	-	-	-	15	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.

Only recently CIDC-Lelystad started with the monitoring of resistance to tylosin and tiamulin in *B. hyodysenteriae* in The Netherlands. The inclusion of this bacterial species in the programme is important because the realistic scenario that this species is becoming resistant to all drugs licensed. Tylosine and tiamulin are included, because they represent all antibiotics used to treat dysentery in pigs. Tylosin is cross resistant with lincomycin and tiamulin with valnemulin. The strains tested are all isolated from animals suffering from swine dysentery at the Animal Health Service in Deventer, The Netherlands. All isolates were typed with PCR at the Animal Sciences Group in Lelystad, The Netherlands. As was expected all strains were resistant to tylosine (and therefore also to lincomycin), and all strains were susceptible to tiamulin (and therefore also to valnemulin).

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 2002.

	MIC distribution (µg/ml)															
***E. coli* (N = 105)**	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R**
Amoxicillin	-	-	-	-	-	-	2,9	21,9	46,7	14,3	1,0	-	13,3	-	-	13,3
Amox/clav. acid	-	-	-	-	-	-	3,8	37,1	39,0	15,2	3,8	-	1,0	-	-	1,0
Cefquinome	-	-	84,8	11,4	1,9	1,0	1,0	-	-	-	-	-	-	-	-	0,0
Cefoperazone	-	-	-	16,2	50,5	13,3	9,5	1,0	1,0	1,9	6,7	-	-	-	-	0,0
Cefuroxime	-	-	-	-	-	-	-	2,9	58,1	37,1	1,0	1,0	-	-	-	1,0
Tetracycline	-	-	-	-	-	1,0	54,3	23,8	6,7	-	-	-	14,3	-	-	14,3
Gentamicin	-	-	-	-	4,8	54,3	28,6	9,5	1,9	1,0	-	-	-	-	-	0,0
Kanamycin	-	-	-	-	-	-	1,0	27,6	54,3	7,6	1,9	7,6	-	-	-	0,0
Neomycin	-	-	-	-	-	-	19,0	57,1	14,3	1,9	-	7,6	-	-	-	7,6
Streptomycin	-	-	-	-	-	-	-	-	18,1	59,0	7,6	1,0	1,0	13,3	-	15,2
Enrofloxacin	-	88,6	10,5	-	1,0	-	-	-	-	-	-	-	-	-	-	0,0
Trim/sulpha	-	-	-	84,8	2,9	1,0	-	-	-	-	-	11,4	-	-	-	11,4
Coliform bacteria (N = 108)	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R**
Amoxicillin	-	-	-	-	-	-	0,9	3,7	2,8	4,6	3,7	5,6	78,7	-	-	84,3
Amox/clav. acid	-	-	-	-	-	-	21,3	43,5	6,5	3,7	3,7	6,5	13,0	1,9	-	21,3
Cefquinome	-	-	83,3	10,2	5,6	-	0,9	-	-	-	-	-	-	-	-	0,0
Cefoperazone	-	-	1,9	8,3	38,9	10,2	8,3	18,5	12,0	0,9	0,9	-	-	-	-	0,0
Cefuroxime	-	-	-	-	-	-	8,3	45,4	17,6	13,9	7,4	7,4	-	-	-	7,4
Tetracycline	-	-	-	-	-	14,8	50,0	17,6	1,9	2,8	1,9	2,8	8,3	-	-	15,7
Gentamicin	-	-	-	24,1	57,4	15,7	2,8	-	-	-	-	-	-	-	-	0,0
Kanamycin	-	-	-	-	-	5,6	43,5	30,6	8,3	1,9	2,8	7,4	-	-	-	0,0
Neomycin	-	-	-	-	14,8	59,3	17,6	4,6	0,9	1,9	-	0,9	-	-	-	0,9
Streptomycin	-	-	-	-	-	-	1,9	38,9	47,2	2,8	0,9	2,8	3,7	1,9	-	8,3
Enrofloxacin	-	77,8	15,7	1,9	3,7	0,9	-	-	-	-	-	-	-	-	-	0,0
Trim/sulpha	-	-	-	82,4	11,1	2,8	-	-	-	-	-	3,7	-	-	-	3,7

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 tested, enrofloxacin and gentamicin. The level of the breakpoint MIC used for kanamycin (≥ 16 µg/ml) caused the difference between resistance percentages for neomycin (≥ 8 µg/ml) and kanamycin. Looking at the MIC distribution of kanamycin, 7,6% of the strains demonstrated reduced susceptibility, which is identical to the R% for neomycin.
The coliform bacteria (28 *Enterobacter ,* 68 *Klebsiella,* 12 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 level of resistance to tetracycline was noteworthy.

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 2002.

	MIC distribution (µg/ml)															
***S. aureus* (N= 110)**	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R**
Penicillin	-	-	90,0	-	-	-	1,8	-	1,8	1,8	4,5	-	-	-	-	10,0
Oxacillin	-	-	2,7	42,7	40,9	12,7	0,9	-	-	-	-	-	-	-	-	0,0
Amox/clav. acid	-	-	-	80,9	9,1	2,7	7,3	-	-	-	-	-	-	-	-	0,0
Cephalothin	-	-	9,1	51,8	32,7	6,4	-	-	-	-	-	-	-	-	-	0,0
Tetracycline	-	-	-	-	0,9	97,3	1,8	-	-	-	-	-	-	-	-	0,0
Kanamycin	-	-	-	-	-	16,4	24,5	49,1	10,0	-	-	-	-	-	-	0,0
Neomycin	-	-	-	7,3	54,5	32,7	5,5	-	-	-	-	-	-	-	-	0,0
Streptomycin	-	-	-	-	-	-	2,7	20,0	47,3	24,5	3,6	0,9	0,9	-	-	1,8
Erythromycin	-	-	-	-	9,1	82,7	7,3	-	-	-	-	0,9	-	-	-	0,9
Lincomycin	-	-	-	-	-	13,6	68,2	14,5	-	-	0,9	2,7	-	-	-	3,6
Pirlimycin	-	-	-	-	19,1	59,1	18,2	1,8	1,8	-	-	-	-	-	-	1,8
Trim/sulpha	-	-	-	57,3	2,7	40,0	-	-	-	-	-	-	-	-	-	0,0
***Coag. neg. staph.* (N = 89)**	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R**
Penicillin	-	-	58,4	7,9	2,2	5,6	7,9	3,4	3,4	2,2	9,0	-	-	-	-	33,7
Oxacillin	-	-	4,5	38,2	28,1	13,5	7,9	3,4	1,1	-	3,4	-	-	-	-	4,4
Amox/clav. acid	-	-	-	64,0	19,1	12,4	3,4	1,1	-	-	-	-	-	-	-	0,0
Cephalothin	-	-	2,3	67,0	19,3	6,8	4,5	-	-	-	-	-	-	-	-	0,0
Tetracycline	-	-	-	25,8	39,3	20,2	2,2	3,4	-	-	-	9,0	-	-	-	9,0
Kanamycin	-	-	-	28,1	12,4	24,7	24,7	5,6	2,2	-	-	2,2	-	-	-	0,0
Neomycin	-	-	-	79,8	14,6	-	4,5	-	-	-	1,1	-	-	-	-	0,0
Streptomycin	-	-	-	-	2,2	10,1	15,7	27,0	22,5	6,7	1,1	4,5	10,1	-	-	14,6
Erythromycin	-	-	-	5,6	28,1	48,3	2,2	3,4	3,4	1,1	3,4	4,5	-	-	-	9,0
Lincomycin	-	-	-	-	3,4	20,2	33,7	9,0	2,2	2,2	5,6	23,6	-	-	-	31,5
Pirlimycin	-	-	-	15,7	43,8	11,2	10,1	10,1	3,4	-	1,1	4,5	-	-	-	9,0
Trim/sulpha	-	-	3,4	47,2	6,7	32,6	4,5	-	1,1	1,1	-	3,4		-	-	5,6

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 spite of the intensive use of antibiotics in the control of bovine mastitis in The Netherlands, the *S. aureus* isolates tested were very susceptible to most antibiotics. 10% of the isolates were penicillinase producers, 3.6% was resistant to lincomycin and 1.8% to the related but more potent lincosamide drug, pirlimycin. The coagulase negative staphylococci were more resistant than *S. aureus*. 33,7% were resistant to penicillin and 4,4% to oxacillin. Resistance to lincomycin was quite common (31,5%), resistance to pirlimycin was substantially lower (9%).

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 2002.

	MIC distribution (µg/ml)															
***S. uberis* (N = 103)**	**0,015**	**0,03**	**0,06**	**0,125**	**0,25**	**0,5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**R**
Penicillin	57,3	1,9	12,6	5,8	10,7	1,9	5,8	-	2,9	1,0	-	-	-	-	-	3,9
Amox/clav. acid	13,6	38,8	7,8	19,4	11,7	5,8	2,9	-	-	-	-	-	-	-	-	0,0
Cephalothin	-	1,0	6,8	35,9	12,6	7,8	24,3	1,0	1,0	6,8	-	1,0	1,9	-	-	2,9
Erythromycin	1,0	6,8	43,7	19,4	4,9	2,9	2,9	2,9	-	-	-	-	15,5	-	-	21,4
Trim/sulpha	-	-	13,6	46,6	25,2	3,9	-	1,9	-	2,9	2,9	1,0	1,9	-	-	8,7
Tetracycline	-	-	-	1,9	24,3	28,2	9,7	1,0	1,9	-	2,9	22,3	6,8	1,0	-	33,0
***S. dysgalactiae* (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	97,2	-	1,9	0,9	-	-	-	-	-	-	-	-	-	-	-	0,0
Amox/clav. acid	93,5	4,7	-	1,9	-	-	-	-	-	-	-	-	-	-	-	0,0
Cephalothin	0,9	0,9	21,5	72,0	2,8	-	1,9	-	-	-	-	-	-	-	-	0,0
Erythromycin	4,7	22,4	57,9	0,9	-	-	-	0,9	-	-	-	-	13,1	-	-	14,0
Trim/sulpha	-	0,9	5,6	85,0	5,6	1,9	-	0,9	-	-	-	-	-	-	-	0,0
Tetracycline	-	-	0,9	-	0,9	0,9	0,9	8,4	11,2	-	-	29,0	47,7	-	-	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.

S. uberis (five of the strains were identified with PCR as *S. parauberis*) was more frequently resistant to erythromycin, cephalothin, penicillin and trim/sulpha than *S. dysgalactiae*. Three *S. uberis* isolates were resistant to cephalothin (MIC 32 – 64 µg/ml) but susceptible to amoxicillin/clavulanic acid. This indicates that the resistance mechanism in these three strains is the production of a ß-lactamase enzyme. This is an uncommon mechanism in streptococci as altered penicillin binding protein is the most commonly described resistance mechanism. In the frequency distribution of the MICs for penicillin, amoxicillin/clavulanic acid and cephalothin several peaks can be observed within the susceptible category. This indicates that also altered affinity for penicillin binding proteins occurs resulting in increased MICs. Resistance to tetracycline was highest in *S. dysgalactiae*.

IV 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 commensally veterinary 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 exposition 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 exposition of animals to antibiotics is also necessary.

Monitoring of resistance in bacteria circulating in should focus on bacteria of public health importance from food animals, considering that the food chain is a major route for transfer of bacteria from animals to humans. Bacterial species to be included in the survey are food-borne pathogens and indicator organisms, preferably isolated from healthy animals at slaughter. The inclusion of animal pathogens is of indirect importance for public health, but still very relevant. These pathogens usually represent a worst-case scenario because they are isolated from diseased animals that were treated with antibiotics. Therefore the surveillance of these strains in animal husbandry can be used for early warning purposes regarding the detection of new emerging resistances.

Well-documented examples of hazards identified, regarding non-human use of antimicrobials, are fluoroquinolone resistance in food-borne pathogens (*Salmonella, Campylobacter*) and vancomycine-resistance in enterococci. Moreover, increasing knowledge exists on the risks for humans related to these hazards. Outbreaks of human nalidixic acid resistant *S.* Typhimurium DT104 were traced back to pig and cattle farms. Also evidence exists for transfer of ceftriaxone-resistant *S.* Newport from cattle to a human patient and of fluoroquinolone-resistant *Campylobacter jejuni* strains from poultry to humans. The consequences for human health of such infections may include increased risk of infection with resistant salmonella's when patients are treated for unrelated reasons, increased hospitalisation rates in patients infected with resistant strains, increased frequency of treatment failures and increased severity of infections or chance of systemic infection and death.

Trends of resistance in the Netherlands

Data from 2002 in the current report indicate a slow overall increase in **flumequine** resistance (all flumequine-resistant strains demonstrate decreased susceptibility to ciprofloxacin) in salmonella's in The Netherlands. This increase is serotype specific. In *S.* Typhimurium DT104 and ft 507, the predominant phage types of Typhimurium, the flumequine resistance levels are low and stable. In *S.* Java, however, a very rapid increase in flumequine resistance was observed from 0% in 2000 to 35% in 2002 in strains from broilers. This in spite of the fact the in The Netherlands in food-animals prudent use of fluoroquinolones is widely promoted since the beginning of the nineties. Obviously the potential effects of restrictive use

policies of fluoroquinolones are dependant on the species and the serotype. The fact that resistance can emerge in high levels in a few years only warrants further restriction on use of quinolones in poultry.

In *S.* Enteritidis relatively high levels of flumequine-resistance were observed, but these are almost entirely limited to strains of pt 1 isolated from human patients. This indicates that these strains are imported from abroad, either by travel or by consumption of imported poultry products. Eggs imported from Spain are documented as the source of these strains.

Fluoroquinolone-resistance in *C. jejuni* strains is stable around 40% in poultry. The resistance percentages in human clinical isolates have slowly increased in the last decade from approximately 20% in 1992 to 32% in 2002. For acquisition of human infections with fluoroquinolone-resistant campylobacters, travel contributed to a higher proportion than domestical acquisition.

Resistance to **cefotaxime** (ESBL-positive) was found, both in human and poultry salmonella's. Also in randomly picked *E. coli* strains from broilers cefotaxime-resistance was present in significant numbers (6,1%). This indicates that although in broilers 3rd generation cefalosporins are not used, other factors exists that contribute to the selection of ESBL-positive strains. This may be the usage of amoxicillin for treatment and prevention of necrotic enteritis.

Resistance to **vancomycin** in *E. faecium* remained stable at low levels both in broilers and in pigs in spite of the fact that glycopeptides have not been used for years. Although macrolide resistance in *E. faecium* strains from pigs seemed to further decrease, resistance to the other growth promoters stabilized after the initial decrease when these products were banned 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.

Usage of antibiotics

The therapeutic use of antibiotics in animals in The Netherlands has increased annually since 1990, while the demographic data show that livestock has decreased. This indicates an intensified consumption, in spite of the general Dutch policy to reduce the use of animal drugs. The consumption of macrolides has increased since 1998, while the consumption of (fluoro)quinolones was stable over the years. The main antibiotic classes used in food producing animals are tetracyclines and trimethoprim-sulphonamide combinations. In pig production 80% and in poultry 100% of the numbers of doses are administered as group medication (via food or drinking water). This implicates a lot of animals to be exposed unnecessary.

Comparison to usage in Denmark and the UK

Comparing the Dutch consumption data with those from Denmark, a country with a similar intensified animal production system, demonstrates that the total consumption of therapeutically licensed antibiotics in food-animals in 2002 is substantially higher in the Netherlands (402 versus 97 tonnes). In the UK, in 2001, 420 tonnes of active ingredient were used therapeutically in food animals. To estimate the actual exposition, these data should be related to the life weight tonnes slaughtered for the three main categories of food animals in these countries (Table 1).

Table 1. Live weight tonnes slaughtered in The Netherlands, Denmark and UK in 2002 (tonnes x 1000).

	NL (source: PVE)	DK (source Danmap 2002)	UK (source VMD 2002)
Cattle	176	170	1,441
Pigs	1,377	1,992	998
Poultry	767	212	2,177
TOTAL	2,320	2,374	4,616

This indicates that the overall consumption of antibiotics in The Netherlands in food-animals was substantially higher than in Denmark and the UK.
Consumption patterns are comparable with the UK but different when comparing Danish situation with the Dutch situation. In Denmark, tetracycline and trimethoprim-sulphonamide combinations contribute only to 35% of total use.

Comparing the resistance data from this report with DANMAP 2002 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 *S.* Typhimurium the resistance levels are clearly higher than in Danish *S.* Typhimurium strains. This is also the case in campylobacters from broilers and from humans, in *E. coli* as indicator organism from broilers and to a lesser extend in *E. faecium.* It may reflect the higher consumption pattern in The Netherlands.

As in DANMAP 2002, 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 2000. 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 this first coordinated report of the surveillance activities on antimicrobial consumption of veterinary licensed drugs and resistance in animal bacteria has provided very useful data. The resistance situation in The Netherlands is slightly less favourable than in DENMARK, a country with a similar food-animal husbandry system.

Based on the results of this report it can be recommended that:
- this coordinated monitoring programme should be continued and should report annually;
- the report of consumption data should be standardised at the international level to improve comparability of the data;
- the DDD-analyses of detailed usage data on practitioners-, or farm level should be improved regarding the representativeness of the selection of practitioners and farms included in the analysis;
- although the major food-animal species in The Netherlands (poultry and pigs) are well examined in the present programme, resistance data on strains from cattle (dairy and meat) should be improved;
- Measures should be taken to reduce the consumption of antimicrobial agents;
- 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, including vegetables irrigated with faecal contaminated water, should be included in the monitoring programme.

V Appendices

Salmonella spp.

A total of 5526 isolates were tested for antimicrobial resistance between 1999-2002 (Table 1). Human isolates (N=2941) 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=508) and cattle, including calves (N=135) 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=570; layers, reproduction animals and eggs, N=328) concerned mainly nonclinical Salmonella infections derived from a diversity of monitoring programs on the farm, slaughterhouses and at retail. In 2001 and 2002 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.).

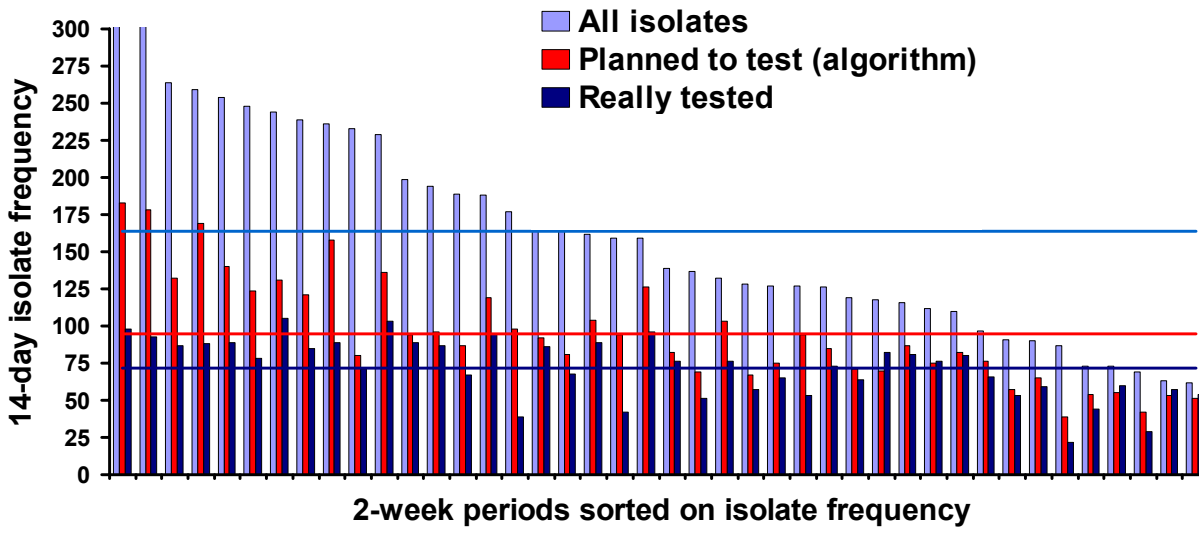
Table 1. Number of Salmonella isolates tested for susceptibility from 1999 – 2002 in the Netherlands.

	Total	1999	2000	2001	2002
Human	2941	674	349	1056	862
Pig	508	31	195	114	168
Cattle	135	18	28	56	33
Chicken (misc.)	316	0	20	154	142
Broilers (faeces/meat)	570	68	100	164	238
Layers/Repro/Eggs	328	93	86	80	69
Other sources	728	22	22	331	353
Total	5526	906	800	1955	1865

Selection procedure of isolates

In 1999 and 2000 selection of the isolates was based, in principal, on those that arrived at the RIVM in the last week of each month and that were derived of a more or less clearly defined source (Table 1). From 2001 onwards, antimicrobial resistance testing was intensified. The new selection procedure went as follows. Within a 14-day period, one isolate was selected for each combination of type (either serotype or phagetype), source (human, animal, food, fodder, etc) and sender of the isolate (institute, laboratory, farm, company, program). The intention of this procedure was to have, from every unit at interest, at least one isolate tested for antimicrobial resistance. This would, theoretically, save the very rare type and source combinations, prevent the selection of multiple isolates of types from the same farm or explosion and considerably reduce redundant sampling of highly frequent salmonella types. Examples of the latter were *S.* Paratyphi B var Java from poultry; *S.* Typhimurium DT104 from humans, cattle and pigs and *S.* Enteritidis Pt4 from humans, layers and eggs. As this selection is stored at minus 80°C this at the same time would guarantee that from each type and event at least one isolate would be conserved for future reference. The proposed selection allowed a very simple automated query into the laboratory information system to produce an Excel-file with the data of the isolates to be selected. The file was sent electronically to CIDC-Lelystad in parallel with the posted isolates. The file including the testing results was returned to the RIVM afterwards.
A two-week period was chosen as this seemed to give the best manageable workload.

Figure 1. Initial number of isolates, those selected automatically by the algorithm and those realised after additional selection by hand. Data concern only those from humans, pigs, cattle and chicken between 2001 and 2002.



Dependent on the season, the numbers of selected isolated might be much higher or lower as manageable. Figure 1 shows the planned 2-weekly selection by the selection-algorithm alone and what selection was realised after additional selection by hand and through unplanned interferences. In table 2 the result of the selection process is summarized: quite a number of the rarer *Salmonella* types are missed indicating that the procedure should be improved.

Table 2. The effect of the automated isolate selection process between 2001-2002 in the different sources. The initial number of isolates, which selection was planned, what has been realised and the number of salmonella types involved (sero- and phagetypes) and number of rare types skipped (various reasons).

	Number of isolates and sero-/phagetypes from 1999-2002				
	Overall	**Human**	**Pig**	**Cattle**	**Chicken/Egg**
All isolates	6869	3167	766	224	2712
Planned selection	3995	2545	328	117	1005
Realised selection	3136	1918	282	89	847
Number of sero-/phagetypes	270	243	66	44	101
Rare types skipped by hand	43	37	8	8	20

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 3 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 (table 2); 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 3. 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

Random selection was achieved by the selection method in 1999 and 2000 testing all isolates in the last week of a month, however this resulted in low numbers (Table 1). The selection method in 2001 and 2002 necessitates that the pattern of resistance within a type is weighted according to its original frequency within a source, to arrive at the overall pattern of resistance within a source. Highly frequent and therefore strongly topped multiresistant types like *S.* Paratyphi B var Java in broilers for example show a strong difference in resistance pattern before and after weighting. Analysing the influence of the missing (rarer) types on the overall resistance pattern of a source proves to be small if the number of isolates tested is not too small.

The effect of differences in the distribution of types between years and sources.

Obviously this plays a role especially in Salmonella. Therefore developments within sources are often described on the serotype level. For example, the increase in flumequine resistance in poultry of the predominant Salmonella Paratyphi B var Java is described separately. In general the effect of type distribution differences within a source over years proves to be small. An important exception are broilers. If the huge increase in broilers of multiresistant

Table 4. Resistance percentages, 1999-2003, if according to the type distribution in humans.

	Human	Pigs	Cattle	Poultry (misc)	Broilers	Layers/Repro/Eggs
Amoxicillin	17,5	16,1	16,1	22,3	19,0	11,8
Cefotaxime	0,6	0,4	0,4	0,4	0,9	0,5
Cefuroxime	0,5	0,4	0,4	0,7	1,1	0,7
Imipenem	0,8	0,6	0,2	1,2	0,2	0,1
Gentamicin	0,8	0,6	0,4	0,4	0,5	0,4
Neomycin	0,2	0,3	0,6	0,1	0,2	0,1
Doxycycline	19,8	21,7	22,7	21,0	16,7	10,9
Trim/Sulpha	7,2	8,0	8,9	8,5	6,7	5,3
Trimethoprim	12,0	9,5	10,5	9,2	8,2	7,4
Ciprofloxacin	0,0	0,0	0,0	0,0	0,0	0,0
Flumequine	5,9	3,9	3,4	6,8	7,2	5,4
Chloramphenicol	11,6	12,0	12,1	12,7	6,5	2,6
Florfenicol	8,4	8,1	8,9	9,4	1,2	0,4

Java over the years is controlled for, the increase in resistance to trimethoprim/suplha and trimetoprim disappears but that of amoxicillin and especially of flumequine still appear to increase strongly.
Table 4 illustrates the Pointe. It shows the pattern of resistance over a three-year period within sources weighted according to the frequency of occurrence of types in humans. Superficially the pattern of resistance now looks almost the same in all sources. However, a difference in resistance of >3% is significant as the number of measurements in this comparison are large, and an explanation in terms of differences in antimicrobial exposure and selection between sources should exist. A clear example of this is the differences seen between broilers, layers and "chickens". The latter probably involving imported poultry products, older animals than broilers and probably involving animals tested for clinical reasons.

Travel related resistance

Salmonella isolates from travel related cases concern about 8% of the cases but several investigations in The Netherlands show that this is an underestimation and is probably closer to about 14%. Table 5 illustrates that a clear difference exists in antimicrobial resistance pattern between travel related and endemic acquired infections.

Table 5. Antimicrobial resistance pattern of travel related and endemic acquired *Salmonella* infections.

1999-2002	Travel	Travel	Non-travel	Non-travel
Amoxicillin	244	11,1	2697	19,0
Cefotaxime	244	0,0	2697	0,6
Imipenem	244	1,2	2697	0,7
Gentamicin	244	1,2	2697	1,6
Neomycin	180	1,1	1738	0,5
Doxycycline	244	16,4	2697	22,3
Cotrimoxazol	244	4,5	2697	7,8
Trimethoprim	244	8,2	2697	12,2
Ciprofloxacin	244	0,8	2697	0,1
Flumequine	244	17,2	2697	6,5
Chloramphenicol	244	4,5	2697	13,1
Florfenicol	244	1,6	2697	8,6
Cefuroxime	180	0,0	1736	0,9

Obviously in human Salmonella infections resistance to (fluoro)quinolones is more travel related. However, domestic acquisition may include consumption of imported animal products including vegetables irrigated with water that is contaminated with faeces. Because of the open market animal products are imported from Brazil and Thailand. Data on contamination of these products with (resistant) bacteria of public health concern are lacking. An example of the import of antimicrobial resistant salmonella's is eggs imported from Spain as source of flumequine resistant *S.* Enteritidis pt 1 infections in humans

Distribution of Salmonella serotypes, in poultry meat products

The surveillance data of VWA-KvW illustrate that in poultry meat products at retail *S.* Java was the predominant serotype isolated in 2001 and 2002. Isolation rates of *S.* Enteritidis, *S.* Hadar and DT104 were reduced in 2002.

Table 6. Distribution of Salmonella serotypes in poultry meat products isolated in 2001 and 2002

	2001	2002
Total number of poultry meat sample examined	1578	1600
Salmonella spp. positive (%)	16,3	13,4
Main serotypes as a fraction of all isolates (%)		
Paratyphi B var. Java	43,2	53,5
Enteritidis	8,2	2,3
Hadar	4,2	0,9
Indiana	11,6	6,5
Infantis	7	7,9
Virchow	3,5	5,6
Typhimurium (DT104)	7,4(7)	7,4(2,8)

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 2002 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 Section of Infectious Diseases the samples were directly 1:10 diluted in buffered peptone solution with 20% glycerol and stored at –20°C pending analysis. *E. coli* and *E. faecium* were isolated within two days after arrival of the samples at CIDC-Lelystad on MacConkey agar and Slanetz and Bartley agar, respectively, by inoculating the plates 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. In 2001 317, and 319 *E. coli* strains, and 285, and 247 *E. faecium* strains were isolated from chickens and pigs, respectively.
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 inoculated on the day the samples arrived at CIDC-Lelystad. From the faecal samples taken from broilers and slaughter pigs at slaughterhouses, as described above, 380 campylobacters were isolated. All campylobacters were typed with PCR to the species level

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.

Brachyspira hyodysenteriae

Brachyspira spp. were isolated from faecal samples of pigs suffering from swine dysentery or sampled at autopsy at the Animal Health Service of Deventer, The Netherlands. Isolates were identified as *B. hyodysenteriae* based on strong ß-hemolysis and indole production. Identification was confirmed by PCR aimed at 23S chromosomal DNA at the Animal Sciences Group, Lelystad.

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 for *Salmonella, Campylobacter, E. coli,* enterococci and staphylococci. The agar dilution test on Mueller Hinton agar was used for *Pasteurella multocida* and *Mannheimia haemolytica*, 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 suspension in saline solution, the trays were incubated micro aerobically in a shaking incubator at 37°C for 24 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.
For *Brachyspira hyodysenteriae* broth micro dilution was used with 48 well trays (VetMic®) prepared by the National Veterinary Institute (SVA), Uppsala, Sweden with Brain Heart Infusion broth supplemented with 10% foetal calf serum. As QC strains *B. hyodysenteriae* ATCC 27164, *Bacteroides fragiles* ATCC 25285, *Eubacterium lentum* ATCC 43055 and *S. aureus* ATCC 29213 were used. The minimum inhibitory concentrations (MIC) 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 7.

Table 7. 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.	*Staphylococcus* spp.	*Brachyspira hyodysenteriae*
Penicillin	-	-	-	-	-	2	0,125	-
Oxacillin	-	-	-	-	-	-	2	-
Amoxicillin	16	16	16	16	16	-	-	-
Amox/clav. acid	16/8	-	16/8	16/8	16/8	16/8	4/2	-
Cephalothin	-	-	-	-	-	16	16	-
Cefuroxime	-	-	-	-	16	-	-	-
Cefoperazone	-	-	-	-	32	-	-	-
Ceftiofur	-	-	-	4	-	-	-	-
Cefquinome	-	-	-	4	4	-	-	-
Cefotaxime	1	-	-	-	-	-	-	-
Imipenem	1	-	-	-	-	-	-	-
Streptomycin	-	8	2000	-	32	-	16	-
Gentamicin	8	8	500	4	8	-	-	-
Kanamycin	-	-	-	-	32	-	16	-
Neomycin	-	8	-	16	16	-	16	-
Spectinomycin	-	-	-	64	-	-	-	-
Tetracycline	-	-	-	4	4	4	4	-
Doxycycline	4	4	4	-	-	-	-	-
Sulphamethoxazole	-	256	-	-	-	-	-	-
Trimethoprim	8	-	-	-	-	-	-	-
Trim/sulphamethoxazole	8/152	8/152	-	4/76	2/38	2/38	2/38	-
Nalidixic acid	-	16	-	-	-	-	-	-
Flumequine	4	-	-	4	-	-	-	-
Enrofloxacin	2	-	-	2	2	-	-	-
Ciprofloxacin	2	2	8	-	-	-	-	-
Chloramphenicol	16	16	32	16	-	-	-	-
Florfenicol	16	-	-	8	-	-	-	-
Nitrofurantoine	-	-	128	-	-	-	-	-
Vancomycin	-	-	16	-	-	-	-	-
Teicoplanin	-	-	16	-	-	-	-	-
Avilamycin	-	-	16	-	-	-	-	-
Bacitracin	-	-	128	-	-	-	-	-
Flavomycin	-	-	16	-	-	-	-	-
Quinu/dalfopristin	-	-	4	-	-	-	-	-
Virginiamycin	-	-	8	-	-	-	-	-
Erythromycin	-	-	4	-	-	0,5	4	-
Tylosin	-	-	-	-	-	-	-	16
Tilmicosin	-	-	32	-	-	-	-	-
Lincomycin	-	-	-	-	-	-	4	-
Pirlimycin	-	-	-	-	-	-	2	-
Tiamulin	-	-	-	-	-	-	-	2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