Associations between antimicrobial use and the prevalence of resistant micro-organisms

Is it possible to benchmark livestock farms based on resistance data?

June 2016

Preface

Over the past few years, the Dutch livestock sector has managed to decrease the amounts of antibiotics used. The implementation of benchmarking systems for livestock farms and veterinarians contributed significantly to this success. The current benchmarking systems are based on a pragmatic approach aimed at identifying differences in antimicrobial usage levels and prescription patterns between livestock farms and veterinarians, respectively, in order to promote prudent usage of antibiotics. The benchmarking thresholds (cut-off values separating the target, signaling and action zones) are set by the expert panel of the Netherlands Veterinary Medicines Authority (SDa). The current thresholds bear no relation to the prevalence of antimicrobial resistance in a particular livestock sector or at a particular livestock farm. The SDa expert panel recently analyzed whether resistance data could serve as the basis for benchmarking thresholds. This report describes the analyses performed and the results of this exercise.

We would like to use this opportunity to thank Prof Dik Mevius, Cindy Dierikx and Kees Veldman for making the Wageningen UR Central Veterinary Institute's (CVI's) antimicrobial resistance monitoring data available and accessible for inclusion in the analyses, and for commenting on the results of the analyses.

We also want to thank José Jacobs and Alejandro Dorado García, DVM, MSc VEE for their valuable contribution to the development of this report.

Our final words of thanks are reserved for the national and international experts who met with the expert panel in June 2015; in alphabetical order: Prof R. Coutinho, Prof J. Dewulf, Prof J. van Dissel, Prof K. Grave, Prof D. Mevius, Prof S. McEwen and Prof M. Scott. The expert panel greatly appreciates their valuable and constructive comments during the early stages of the drafting of this report.

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Summary

The expert panel analyzed the relationship between antimicrobial use in the Dutch livestock sector and the prevalence of antimicrobial-resistant micro-organisms in livestock. For its analyses, the expert panel examined usage data collected through random sampling of Dutch livestock farms, as published by LEI Wageningen UR for the 2004-2011 period, as well as data regarding the Dutch livestock sector as a whole, as recorded by the SDa for the years 2011 to 2015. The resistance data used in the analyses were originally recorded by Wageningen UR's Central Veterinary Institute as part of the Dutch antimicrobial resistance monitoring program. These data were collected through sampling performed by the Netherlands Food and Consumer Product Safety Authority. The resistance data pertain to the years 2009 to 2014. Data on antimicrobial use in Dutch livestock revealed a downward trend. This enabled the expert panel to assess how the decline in usage levels affected the prevalence of antimicrobial-resistant bacteria over the years. In this report, the expert panel discusses its findings in the light of the scientific literature available on antimicrobial resistance.

The expert panel performed its analyses to find out whether information on associations between antimicrobial use and the prevalence of resistance to antibiotics can serve as a basis for benchmarking thresholds. This report therefore rather cautiously refers to such thresholds as "resistance-informed benchmarking thresholds". The expert panel analyzed the data to find answers to the research questions set out below, and answered the questions as follows:

To what extent is the decline in antimicrobial usage levels achieved over the past few years associated with a reduction in the prevalence of antimicrobial-resistant bacteria? The decline in antimicrobial usage levels was associated with reductions in the prevalence of antimicrobial-resistant *E. coli* in fecal samples from veal calves, pigs and broilers. The decrease in prevalence varied widely between species and depended on the type of resistance concerned. The veal farming sector achieved a 37.4% reduction in total usage of antibiotics over the observation period analyzed. This reduction was associated with a 26% decline in the prevalence of resistance to one or more classes of antibiotics (*overall resistance*). Since this sector already started to reduce its usage levels in 2007, the total decline over the past few years will exceed the decline reported for the observation period. It should be noted, however, that the veal farming sector's decrease in resistance levels is confounded to some extent by a change in the sampling strategy during the observation period. The pig farming sector managed to reduce its total use of antibiotics by 54%, associated with a more modest decrease in the prevalence of resistance to one or more classes of antibiotics (22%). The broiler farming sector recorded a smaller reduction in the level of resistance to one or more classes of antibiotics (8%), even though total antimicrobial use in this sector decreased by 57%. Potential reasons for this discrepancy are addressed in this report. According to the expert panel, further investigation is required to establish effective measures for reducing resistance issues in the years to come.

To what extent does the prevalence of antimicrobial-resistant bacteria vary between the various livestock sectors? Many *E. coli* strains are multidrug-resistant, i.e. resistant to three or more classes of antibiotics. According to this definition, 4.5% of strains isolated from dairy cattle in 2014 were multidrug-resistant. The prevalence of multidrug-resistant strains was quite different in the other livestock sectors, with 45.0% for the veal farming sector, 53.7% for the pig farming sector and 75.4% for the broiler farming sector. Considering the samples were taken from healthy animals, the multidrug-resistance rates in these three livestock sectors were high. The low prevalence of multidrug-resistance in the dairy farming sector can be explained by this sector's low antimicrobial usage levels and its practice of using selective rather than whole-herd treatment regimens. In the dairy farming sector, variations over time could be due to the fact that pooled fecal samples (from animals of all ages) were used for some of the tests.

The broiler farming sector managed to reduce its resistance levels substantially over the past few years, with the prevalence of resistance to third- and fourth-generation cephalosporins showing the biggest improvement. The fact that these antibiotics are no longer used at hatcheries presumably was the main driver of this improvement. The decline in usage of broad-spectrum penicillins as a second-choice agent at broiler farms probably was another contributing factor. A prior policy decision to heavily restrict usage of third-choice agents did not yet markedly decrease the prevalence of resistance to quinolones and fluoroquinolones. Compared to the other livestock sectors, the proportion of *E. coli* isolates resistant to fluoroquinolones in the broiler farming sector was still high (46.4%).

Statistically significant correlations were found between the amounts of antibiotics used (total and antimicrobial-specific usage levels) and the prevalence of antimicrobial-specific resistance in *E. coli* isolates. In many cases, antimicrobial-specific resistance in *E. coli* isolates was more strongly associated with total usage levels than with antimicrobial-specific usage levels. This might have been due to the presence of co-resistant or cross-resistant micro-organisms.

Do the results indicate that associations between antimicrobial use and antimicrobial-resistant bacteria can serve as the basis for benchmarking thresholds? As yet, the associations between antimicrobial use and antimicrobial resistance revealed by the analyses do not indicate a particular usage level below which the level of resistance is equal to the background level (threshold value), although resistance thresholds regarding the relationship between use of and resistance to antibiotics could potentially be used to derive new benchmarking thresholds. Current scientific literature also does not allow for such threshold values to be derived. Furthermore, it is not yet possible to define an "acceptable resistance level". This would require information on resistance-related risks such as public health risks, and currently available data do not allow for quantification of such risks. If an acceptable resistance level were to be determined, the corresponding antimicrobial usage level could serve as the basis for benchmarking values. As yet, however, no acceptable resistance level has been defined.

Conclusions

Based on the findings in this report, the expert panel concludes that the observed associations between reductions in antimicrobial use and reductions in the prevalence of antimicrobial resistance in *E. coli* isolates, strongly suggest that an additional reduction in usage levels will further decrease the prevalence of resistant micro-organisms.

However, these associations lack the strength and specificity to facilitate predictions as to how resistance levels will develop in the event of further usage level reductions. With regard to juvenile meat-producing animals in particular (veal calves, broilers, pigs), levels of resistance to several classes of antibiotics are still high and co-resistance has been observed. The expert panel therefore wants the three livestock sectors concerned to further reduce their usage levels for all classes of antibiotics. This conclusion is based on the precautionary approach to public health, with consideration of preconditions regarding animal health and animal welfare.

Recommendations

No benchmarking thresholds indicating an acceptable resistance level can be derived from currently available data. The existing pragmatic benchmarking approach therefore remains crucial for providing insight into the amounts of antibiotics used in the Dutch livestock sector. According to the expert panel, each livestock sector should continue to focus on the livestock farms within the action zone (red) and the signaling zone (orange) in order to further reduce its antimicrobial usage levels in the years to come. This is in line with the recommendations included in the SDa report "Usage of Antibiotics in Agricultural Livestock in the Netherlands in 2014", which was published in September 2015. In order to further reduce the amounts of antibiotics used, it is necessary to find out why certain livestock farms have high usage levels. Additional investigation is therefore required to identify determinants of antimicrobial use and to define appropriate interventions. The parties concerned should subsequently implement these interventions and evaluate their effect.

The current Dutch resistance monitoring system is based on EU legislation, and predominantly involves testing of livestock isolates collected at slaughterhouses. The test results give an idea of the effects antimicrobial use has on the prevalence of resistant intestinal bacteria in the various types of livestock. When this information is combined with data on the prevalence of resistance in bacteria isolated from meat, it provides insight into the risk of resistant strains being transferred to humans through direct contact, the environment or the food chain. These data are, however, just an indication of the prevalence of resistance at individual livestock farms. Although the process of testing livestock isolates collected at slaughterhouses can quite accurately determine the risk of resistant bacteria spreading to consumers through the food chain, it does not take account of other relevant forms of transmission. After all, work-related transmission, transmission from people with work-related high exposure levels to their family members or the general population, environmental transmission, and transmission within and between individual livestock farms may also be involved. At the moment, sampling at slaughter houses does not give a proper indication of how usage and resistance levels are correlated.

The expert panel therefore proposes that regular surveys be conducted to assess resistance-related issues and developments at individual livestock farms. Such surveys should be conducted for each of the livestock sectors. They should serve to obtain antimicrobial usage data from individual livestock farms, which data could then be combined with resistance data derived from the testing of fecal samples. The additional information would help determine the relationship between usage and resistance levels, and might at a later date lead to better substantiated benchmarking thresholds. As a final recommendation, the expert panel suggests that resistance patterns observed at livestock farms in the red and orange zones should be communicated to individual livestock farmers and the livestock sectors concerned. After all, these patterns show how usage and resistance levels are related for livestock farmers in these benchmarking zones.

Current policy mainly focuses on usage of first-, second- and third-choice agents, with application of third-choice agents being restricted to human medicine as much as possible. The classification of first- and second-choice agents is largely based on the recent emergence of resistant ESBL-producing organisms. Once new types of resistance emerge, this classification may have to be updated accordingly. Even first-choice agents cannot be deemed 100% risk free. The expert panel therefore recommends to continue reducing usage of all antibiotics, first-choice agents included.

Introduction

The SDa promotes transparency regarding the usage of antibiotics in agricultural livestock. To this end the SDa monitors usage data of the main livestock sectors, assesses sales figures, and benchmarks usage levels of livestock farms as well as prescription patterns of veterinarians. When the benchmarking system for usage of antibiotics at Dutch livestock farms was introduced in 2011, it was not based on preventing emergence and spread of resistant micro-organisms. Due to the lack of quantitative data on associations between antimicrobial use and antimicrobial resistance, the benchmarking system was based on a pragmatic approach. Usage levels turned out to vary distinctly between individual livestock farms. When a benchmarking system for veterinarians was implemented, veterinarians' prescription patterns also showed substantial variations. It was not clear what caused these variations. When defining the benchmarking thresholds, the expert panel therefore assumed livestock farms with high usage levels would be able to bring their usage levels in line with those of other livestock farms within the livestock sector concerned. The current benchmarking thresholds are based on this assumption, and are intended to reduce differences in usage levels between the livestock farms within particular livestock sectors and differences in prescription patterns between veterinarians.

Following years of declining usage levels, the parties involved, i.e. the livestock sectors, veterinarians and the authorities, would now like to know how antimicrobial use and the prevalence of resistant micro-organisms are related. In light of this, they would prefer a benchmarking system that is based on data regarding the prevalence of antimicrobial resistance. The expert panel therefore decided to examine peer-reviewed articles on this subject and analyze associations between antimicrobial use in the various Dutch livestock sectors and the prevalence of resistant micro-organisms. For its analyses, the expert panel used data provided by the Central Veterinary Institute (CVI) in its annual MARAN reports.

When starting its analyses, the expert panel did not know whether resistance data can be directly translated into benchmarking thresholds. This report therefore rather cautiously refers to such thresholds as "resistance-informed benchmarking thresholds". With its analyses, the expert panel set out to answer the following questions:

1. To what extent is the decline in antimicrobial usage levels achieved over the past few years associated with a reduction in the prevalence of antimicrobial-resistant bacteria?
2. To what extent does the prevalence of antimicrobial-resistant bacteria vary between the various livestock sectors?
3. Do the results indicate that associations between antimicrobial use and antimicrobial-resistant bacteria can serve as the basis for benchmarking thresholds?

In order to answer these questions, the expert panel examined antimicrobial usage and resistance data collected by LEI, the SDa and CVI over the past few years.

In June 2015, the expert panel met with several national and international experts (see Appendix 1). During this meeting, the experts could comment on the expert panel's analyses. The analyses will also be submitted to a scientific journal for publication. This report contains a concise description of the analyses performed by the expert panel without all of the technical and scientific details, and a discussion of any implications for the SDa's benchmarking system.

Emergence and spread of antimicrobial resistance

In the occurrence of antimicrobial resistance, several distinct phases can be distinguished: (1) emergence, (2) selection, (3) spread, (4) persistence, and (5) reduction. Each of the phases is briefly addressed below.

(1) Emergence of resistance refers to the process during which bacteria susceptible (sensitive) to antibiotics become insusceptible (resistant) to antibiotics. Resistance might emerge as a result of mutations or through so-called "horizontal gene transfer" of resistance genes. (2) Subsequent exposure to antibiotics facilitates selection of the resistant bacteria. Since the antibiotics will kill or inhibit susceptible bacteria occupying the same niche as the resistant bacteria, this creates opportunities for the resistant bacteria to grow and multiply. Due to their acquired resistance genes or mutations, resistant bacteria differ from the "wild-type" bacteria, i.e. the bacteria that originally occupied the niche, prior to the selective pressure introduced by usage of antibiotics. (3) Resistant bacteria can subsequently spread to other hosts (humans and/or animals) and/or into the environment. (4) Occurrence of selection and spread is highly dependent on the usage of antibiotics. Whether resistant bacteria remain present in their initial or - if spread has occurred - new host (persistence), in part depends on the selective pressure exerted by antibiotics. Usage of antibiotics favors resistant micro-organisms over susceptible micro-organisms. (5) Resistance rates in a bacterial population can drop if the growth of susceptible bacteria exceeds the growth of resistant bacteria (due to a competitive advantage of the former), or if the bacteria lose their resistance genes or point mutations. In its monitoring reports, CVI uses the term "non-wild type" to refer to resistant bacteria.

In short: exposure to antibiotics drives the emergence, spread and persistence of resistance. This complicates any association between antimicrobial use and antimicrobial resistance, with the dynamic nature of resistance representing an additional challenge. Following selection, the resistant bacteria will grow and multiply at a particular speed (i.e. the increase in the number of bacteria per unit of time). The bacterial growth rate depends on the type of bacteria (slow-growing or fast-growing bacteria), the type of resistance (and the extent to which it affects bacterial growth), and the environment (nutrients, temperature). Consequently, it is not possible to define correlations between antimicrobial use and antimicrobial resistance with a univocal, model-based approach. For its analyses, the expert panel therefore decided to examine empirical relationships between resistance and usage data, and developments in these relationships over time. In doing so, the expert panel actually analyzed the overall effect of all statistical associations between the underlying variables. So rather than analyzing the specific contribution of each underlying variable, the expert panel actually analyzed the overall effect of all statistical correlations between these variables.

The data and the analyses

Resistance data

Data on the prevalence of resistant strains were provided by the Central Veterinary Institute (CVI). CVI performs annual susceptibility tests on *E. coli* isolates collected in the four livestock sectors (the dairy, veal, poultry and pig farming sector) as part of a Dutch monitoring program.[1] This monitoring program was implemented to detect and monitor population-level changes in antimicrobial resistance by means of testing randomly selected samples for the various livestock sectors. These national monitoring efforts are part of a European harmonized monitoring program.[2] Resistance data for the poultry and pig farming sectors have been reported since 1998, and resistance data for the veal and dairy farming sectors since 2005. Until 2011, samples from veal calves were collected at calf fattening farms. As of 2012, these samples are obtained at slaughter houses. Except for the samples collected in 2010 and 2011, all dairy cattle samples were pooled farm-level samples. In 2010 and 2011, samples from individual animals were used instead, collected at slaughter houses. This period overlaps a brief period during which higher resistance levels were observed. This probably was associated with smaller sample sizes used during that period. The MARAN reports provide more details on the design of CVI's monitoring process (sampling at slaughter houses).[1] Resistant strains were identified based on the Minimal Inhibitory Concentrations (MICs) for *E. coli* isolates, with epidemiological cut-off values[*]. These non-clinical cut-off values are more objective than clinical breakpoints and better suited to monitor emergence of newly acquired resistance.

Monitoring of antimicrobial use by LEI and the SDa

Since 2011, the SDa reports usage data for each of the four livestock sectors based on the delivery records for 13 classes of antibiotics. The most recently reported data, pertaining to 2014, were also included in the analyses. When calculating the amounts of antibiotics used, the SDa uses different measures for usage at individual livestock farms ($DDDA_F$) and usage at a national level ($DDDA_{NAT}$).[3] It uses the ESVAC system[**] for reporting the defined daily dose for animals (DDDA). Virtually all Dutch livestock farms are included in the SDa data. Data up to 2011 were provided by the Dutch Agricultural Economics Institute (LEI) of Wageningen University (WUR). These LEI data were based on stratified random samples of livestock farms. For the pig farming sector, reports normally include separate usage levels for pig fattening farms and sow/piglet farms. In the current report, however, usage data for fattening pigs and sows/piglets are pooled in order to provide insight into the amounts of antibiotics used during a pig's entire life span. Data from Statistics Netherlands (CBS) were used to determine the average proportion of pigs that was included in each of the production types[***] during the 2000-2010 period (51% fattening pigs, 49% sows/piglets). By using these percentages as weighting factors, the two production types could be merged to produce a single usage level for the pig farming sector. For the years 2011 to 2014,

*http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_SOPs/EUCAST_definitions_of_clinical_breakpoints_and_ECOFFs.pdf
**http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000302.jsp&mid=WC0b01ac0580153a00&jsenabled=true
***http://statline.cbs.nl/Statweb

data for the pig farming sector as a whole were readily available, and therefore did not have to be calculated using weighting factors.

Statistical considerations

More detailed information on the analyses presented in this report is provided in a scientific article that will be published seperately.[4] This section therefore only includes a general outline of the statistical methods used. The methods are in line with methods proposed or used by other organizations in several recent reports:[5, 6]

- long-term national trends in antimicrobial use per livestock sector were linked to resistance data collected annually by CVI;
- both total usage data and usage data for several specific classes of antibiotics were linked to resistance data;
- the following types of antimicrobial resistance were analyzed: resistance to penicillins (AMP), tetracyclines (TET), sulfonamides (SMX), trimethoprim (TMP), fluoroquinolones (CIP), quinolones (NAL), amphenicols (CHL), third- and fourth-generation cephalosporins (FOT), and aminoglycosides (STM, GEN) (see Table 1);

Table 1. Classes of antibiotics with the corresponding first-, second- or third-choice designations and types of resistance

Class of antibiotics	1st, 2nd or 3rd choice	Resistance
Amphenicols	1st	CHL
Aminoglycosides	2nd	STM, GEN
1st- and 2nd-generation cephalosporins	2nd	
3rd- and 4th-generation cephalosporins	3rd	FOT
Quinolones	2nd	
Fluoroquinolones	3rd	CIP
Macrolides/lincosamides	1st or 2nd	
Penicillins	1st or 2nd	AMP
Polymyxins	2nd	
Tetracyclines	1st	TET
Trimethoprim/sulphonamides	1st	TMP, SMX

- for the current analyses, only isolates of one indicative micro-organism were examined: isolates of the bacterium *E. coli*;
- several statistical techniques were used, which required various assumptions: annual observations were assumed to be 100% independent over time (non-temporal), annual observations were adjusted for correlations over time, and analyses with exposure lagging were conducted (current level of resistance linked to last year's usage level). None of these alternative analyses changed the overall findings, which is why they have not been included in this report.

Data analysis results

Associations between antimicrobial use and antimicrobial resistance

The data in Table 2 quantify changes in usage and resistance levels between 2009 and 2014. For most of the commonly used antibiotics, the strongest declines in usage levels were observed in the pig and broiler farming sectors. In the broiler farming sector, total antimicrobial use decreased by 57%, with tetracyclines and quinolones showing the strongest decline (70% and 68%, respectively). In the pig farming sector, total antimicrobial use decreased by 54%, with trimethoprim/sulphonamides and tetracyclines showing the strongest decline (63% and 59%, respectively). With a 37.4% reduction in total use, the reduction in antimicrobial use achieved in the veal farming sector was smaller than the reduction observed for the pig farming sector.

The smallest improvement in resistance levels over the 2009-2014 period was observed in the broiler farming sector. This sector reduced its *overall* level of resistance (i.e. resistance to one or more classes of antibiotics) by just 8%, and its level of resistance to tetracyclines by 31%. The pig farming sector achieved larger improvements. It managed to reduce its *overall* resistance level by 22%, and its level of resistance to penicillins by 47%. The veal farming sector achieved resistance level reductions similar to those in the pig farming sector, with a 26% reduction in *overall* resistance and a 46% reduction in resistance to penicillins, although it did not manage to decrease its usage levels as well as the pig farming sector. As mentioned before, it has to be taken into account that the veal farming data are confounded by a change in the sampling process. As of 2012, sampling takes place at slaughterhouses rather than calf fattening farms, which means intervals between final usage of antibiotics and the time of sampling for resistance monitoring have increased. It is likely that as a result of this, the levels of resistance measured since 2012 were lower than in the years before. Due to this change in sampling method, the actual decline in resistance levels is probably smaller than the reported data suggest.

All livestock sectors managed to reduce the use of fluoroquinolones and third- and fourth-generation cephalosporins to zero or just above by 2014. This improvement was associated with substantial resistance level reductions:

- the broiler farming sector reduced its cefotaxime (third- and fourth-generation cephalosporins) and ciprofloxacin (fluoroquinolones) resistance levels by 84% and 19%, respectively;
- the pig farming sector managed to reduce its cefotaxime (third- and fourth-generation cephalosporins) and ciprofloxacin (fluoroquinolones) resistance levels by 86% and 100%, respectively;
- the veal farming sector reduced its cefotaxime (third- and fourth-generation cephalosporins) and ciprofloxacin (fluoroquinolones) resistance levels by 41% and 64%, respectively;
- for the dairy farming sector, no resistance to fluoroquinolones was recorded for 2014. As regards third- and fourth-generation cephalosporins, this sector achieved a 75% reduction in cefotaxime resistance.

Table 2. Changes in antimicrobial use and antimicrobial resistance between 2009 and 2014 for the various livestock sectors.

Livestock sector	Antimicrobial use ($DDDA_{NAT}$)			Prevalence of antimicrobial resistance (%)			Absolute change from 2009 to 2014		Relative change from 2009 to 2014	
	Class of antibiotics	2009	2014	Type of resistance	2009	2014	Antimicrobial use ($DDDA_{NAT}$)	Antimicrobial resistance (%)	Antimicrobial use ($DDDA_{NAT}$)	Antimicrobial resistance (%)[a]
Broiler farming sector	Overall	36.8	15.8	*Overall*[b]	87.6	80.6	-21.0	-7.0	-57.1	-8.0
	Tetracyclines	5.6	1.7	TET	61.9	42.4	-3.9	-19.4	-69.8	-31.4
	Penicillins	14.3	9.9	AMP	73.2	62.1	-4.4	-11.1	-30.5	-15.2
	Trimethoprim/ sulfonamides	2.2	1.3	TMP	62.2	44.6	-0.8	-17.6	-37.7	-28.4
				SMX	71.8	52.5				
	Amphenicols	0.0	0.0	CHL	23.7	13.5	0.0	-10.2	0.0	-42.9
	Fluoroquinolones	0.5	0.2	CIP	57.4	46.4	-0.3	-11.0	-64.7	-19.1
	Quinolones	6.7	2.1	NAL	57.4	44.6	-4.5	-12.8	-68.0	-22.3
	3rd- and 4th-gen. cephalosporins	0.0	0.0	FOT	17.9	2.9	0.0	-15.0	0.0	-83.7
	Aminoglycosides	0.0	0.0	STM	67.4	n.a.	0.0	n.a.	0.0	n.a.
				GEN	8.6	6.4				
Pig farming sector	Overall	20.5	9.5	*Overall*[b]	80.4	63.0	-11.0	-17.4	-53.6	-21.6
	Tetracyclines	10.7	4.3	TET	67.6	49.2	-6.4	-18.3	-59.4	-27.1
	Penicillins	2.8	2.1	AMP	44.9	24.0	-0.7	-21.0	-25.9	-46.6
	Trimethoprim/ sulfonamides	3.6	1.3	TMP	53.7	30.9	-2.2	-22.8	-62.6	-42.5
				SMX	61.8	41.3				
	Amphenicols	0.0	0.2	CHL	11.5	12.0	0.1	0.5	278.6	4.4
	Fluoroquinolones	0.0	0.0	CIP	7.1	0.0	0.0	-7.1	-100.0	-100.0
	Quinolones	0.0	0.1	NAL	7.1	0.3	0.0	-6.8	45.8	-96.4
	3rd- and 4th-gen. cephalosporins	0.1	0.0	FOT	3.7	0.5	-0.1	-3.2	-100.0	-86.3
	Aminoglycosides	0.0	0.0	STM	62.5	n.a.	0.0	n.a.	0.0	n.a.
				GEN	3.0	3.6				
Veal farming sector	Overall	33.8	21.2	*Overall*[b]	66.1	49.0	-12.7	-17.1	-37.4	-25.9
	Tetracyclines	17.8	10.7	TET	59.1	44.5	-7.1	-14.5	-40.0	-24.6
	Penicillins	1.5	2.2	AMP	41.5	22.3	0.7	-19.3	44.3	-46.4
	Trimethoprim/ sulfonamides	3.6	2.1	TMP	37.4	22.3	-1.5	-15.2	-41.4	-40.5
				SMX	45.0	28.1				
	Amphenicols	0.6	1.5	CHL	22.2	13.4	0.9	-8.9	145.2	-39.9
	Fluoroquinolones	0.9	0.0	CIP	18.1	6.5	-0.8	-11.6	-97.7	-64.1
	Quinolones	0.2	0.5	NAL	18.7	5.8	0.3	-12.9	133.3	-68.9
	3rd- and 4th-gen. cephalosporins	0.4	0.0	FOT	1.8	1.0	-0.4	-0.7	-100.0	-41.4
	Aminoglycosides	0.1	0.3	STM	47.4	n.a.	0.3	n.a.	580.0	n.a.
				GEN	6.4	3.8				

Dairy farming sector	Overall	5.8	3.3	*Overall*[b]	23.1	4.9	-2.5	-18.3	-43.0	-79.0
	Tetracyclines	0.6	0.4	TET	18.4	3.0	-0.2	-15.4	-37.1	-83.8
	Penicillins	2.8	2.0	AMP	11.8	1.5	-0.8	-10.3	-27.4	-87.3
	Trimethoprim/ sulfonamides	0.2	0.2	TMP	12.5	0.0	0.0	-12.5	14.3	-100.0
				SMX	16.2	2.6				
	Amphenicols	0.0	0.1	CHL	5.9	1.1	0.0	-4.8	100.0	-81.0
	Fluoroquinolones	0.1	0.0	CIP	4.5	0.0	-0.1	-4.5	-100.0	-100.0
	Quinolones	0.0	0.0	NAL	5.9	0.0	0.0	-5.9	0.0	-100.0
	3rd- and 4th-gen. cephalosporins	0.8	0.0	FOT	1.5	0.4	-0.8	-1.1	-100.0	-74.6
	Aminoglycosides	0.0	0.0	STM	16.9	n.a.	0.0	n.a.	0.0	n.a.
				GEN	5.9	0.4				

Types of antimicrobial resistance: AMP ampicillin; TET tetracycline; SMX sulfamethoxazole; TMP trimethoprim; CIP ciprofloxacin; NAL nalidixic acid; CHL chloramphenicol; FOT cefotaxime; STM streptomycin; GEN gentamicin.

[a] Defined as resistance to one or more classes of antibiotics. Green cells indicate declining levels, with darker shades of green corresponding to steeper declines.

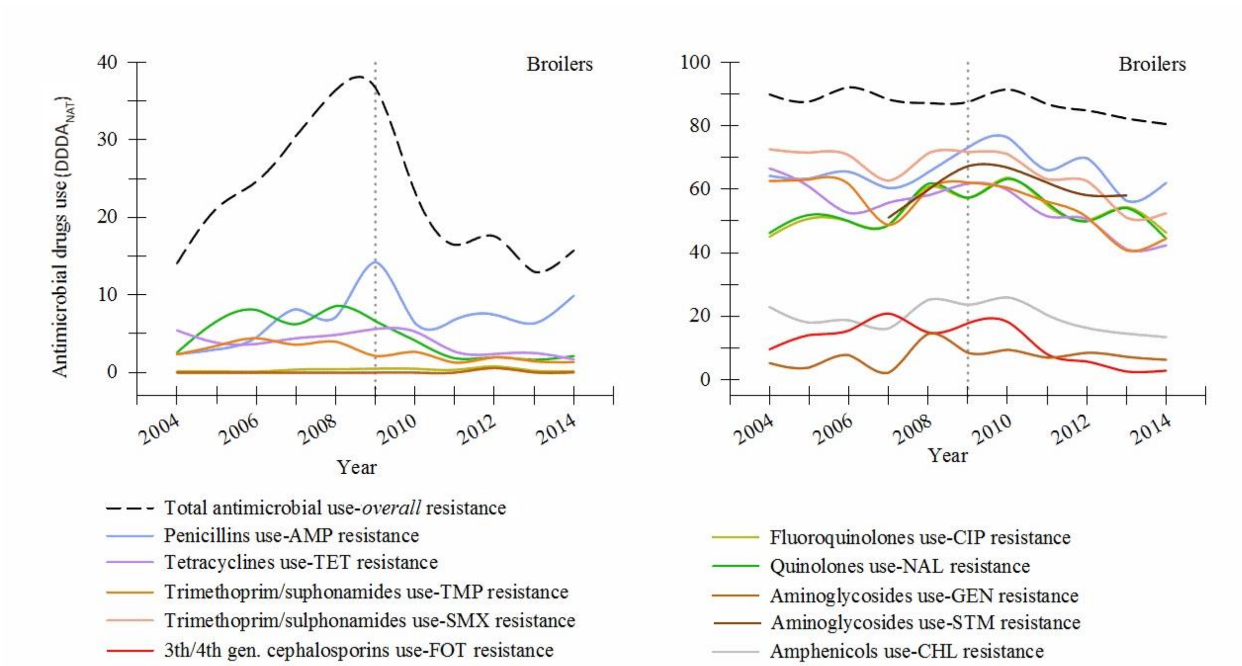
[b] *overall* resistance refers to resistance to at least one of the antimicrobial agents (i.e. excluding fully susceptible isolates).

Figures 1a-d depict trends in antimicrobial use and antimicrobial resistance over the 2004-2014 period. In the broiler and pig farming sectors, usage of antibiotics increased until 2009 and then decreased markedly up to 2014. Resistance levels in these sectors broadly correspond to the usage level patterns. For the veal farming sector, a decrease in both antimicrobial use and the prevalence of resistance in *E. coli* isolates can be observed. However, as mentioned above, this trend in resistance levels has been affected by a sudden change in the sampling process in 2012. Due to this change, the estimated reductions in resistance levels are not deemed to be a true reflection of the actual decrease over time. In the dairy farming sector, usage levels were low throughout the observation period, as were resistance levels. Application of a different sampling method caused antimicrobial resistance to peak between 2008 and 2010.

Although usage patterns differed between the various livestock sectors, all sectors reported relatively high usage levels for tetracyclines, penicillins, trimethoprim and sulfonamides. In the broiler farming sector, use of quinolones was relatively high as well.

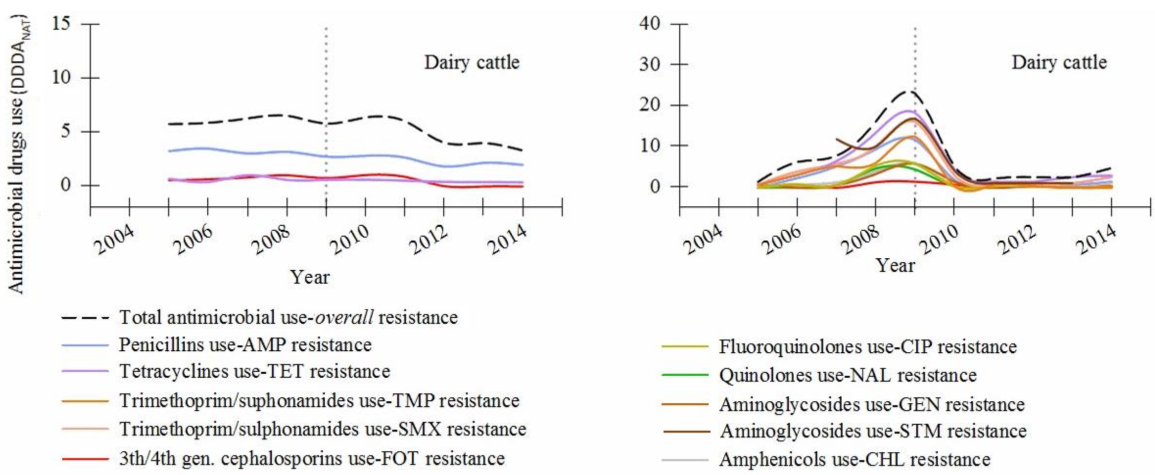
Figures 1a-d. Trends in antimicrobial use and resistance levels for the four livestock sectors,[4] with the year 2009 indicated by a gray line. The Dutch government uses 2009 as the reference year for monitoring trends in antimicrobial usage levels.

a.



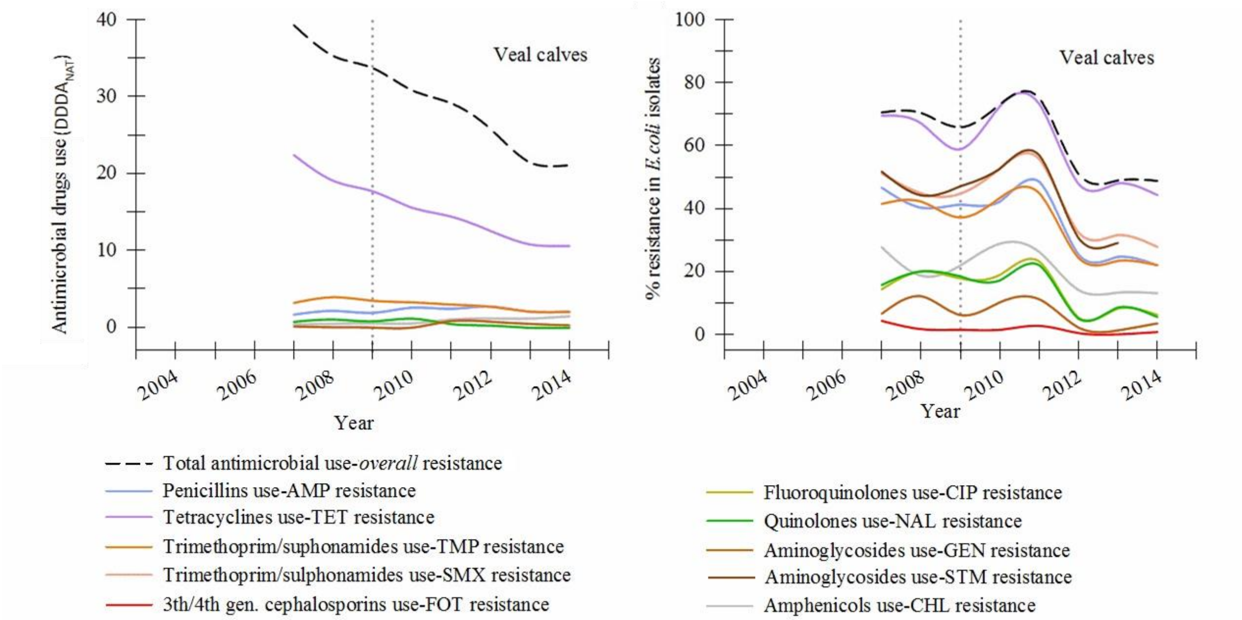
Types of antimicrobial resistance: AMP ampicillin; TET tetracycline; SMX sulfamethoxazole; TMP trimethoprim; CIP ciprofloxacin; NAL nalidixic acid; CHL chloramphenicol; FOT cefotaxime; STM streptomycin; GEN gentamicin

b.



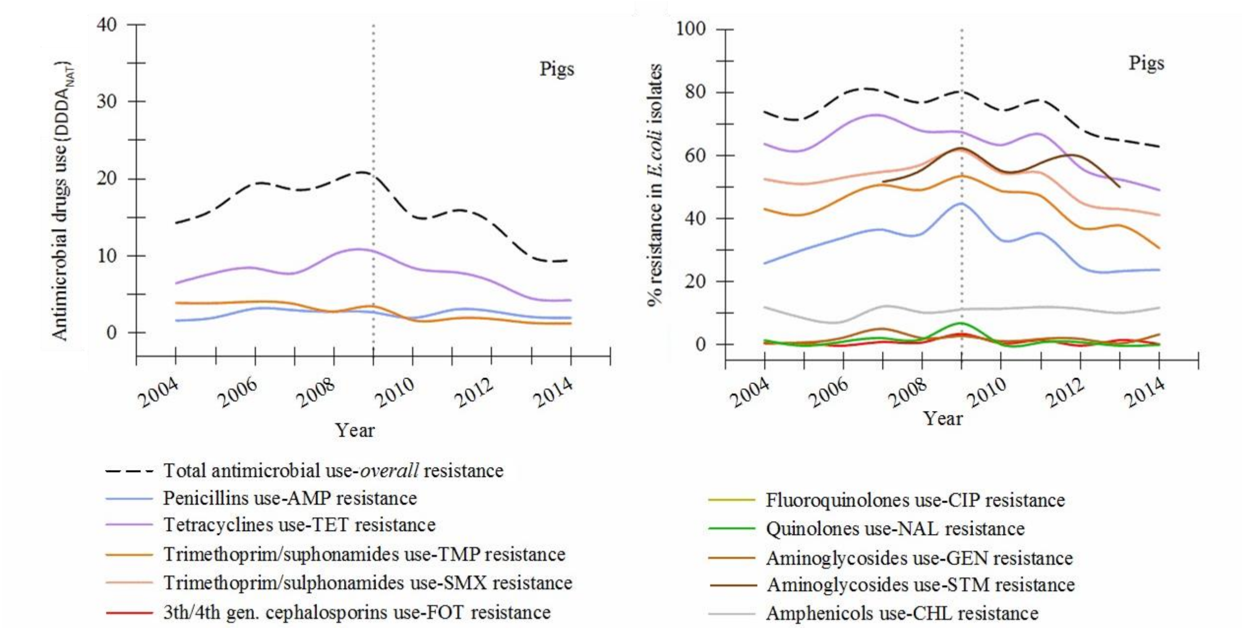
Types of antimicrobial resistance: AMP ampicillin; TET tetracycline; SMX sulfamethoxazole; TMP trimethoprim; CIP ciprofloxacin; NAL nalidixic acid; CHL chloramphenicol; FOT cefotaxime; STM streptomycin; GEN gentamicin

c.



Types of antimicrobial resistance: AMP ampicillin; TET tetracycline; SMX sulfamethoxazole; TMP trimethoprim; CIP ciprofloxacin; NAL nalidixic acid; CHL chloramphenicol; FOT cefotaxime; STM streptomycin; GEN gentamicin

d.



Types of antimicrobial resistance: AMP ampicillin; TET tetracycline; SMX sulfamethoxazole; TMP trimethoprim; CIP ciprofloxacin; NAL nalidixic acid; CHL chloramphenicol; FOT cefotaxime; STM streptomycin; GEN gentamicin

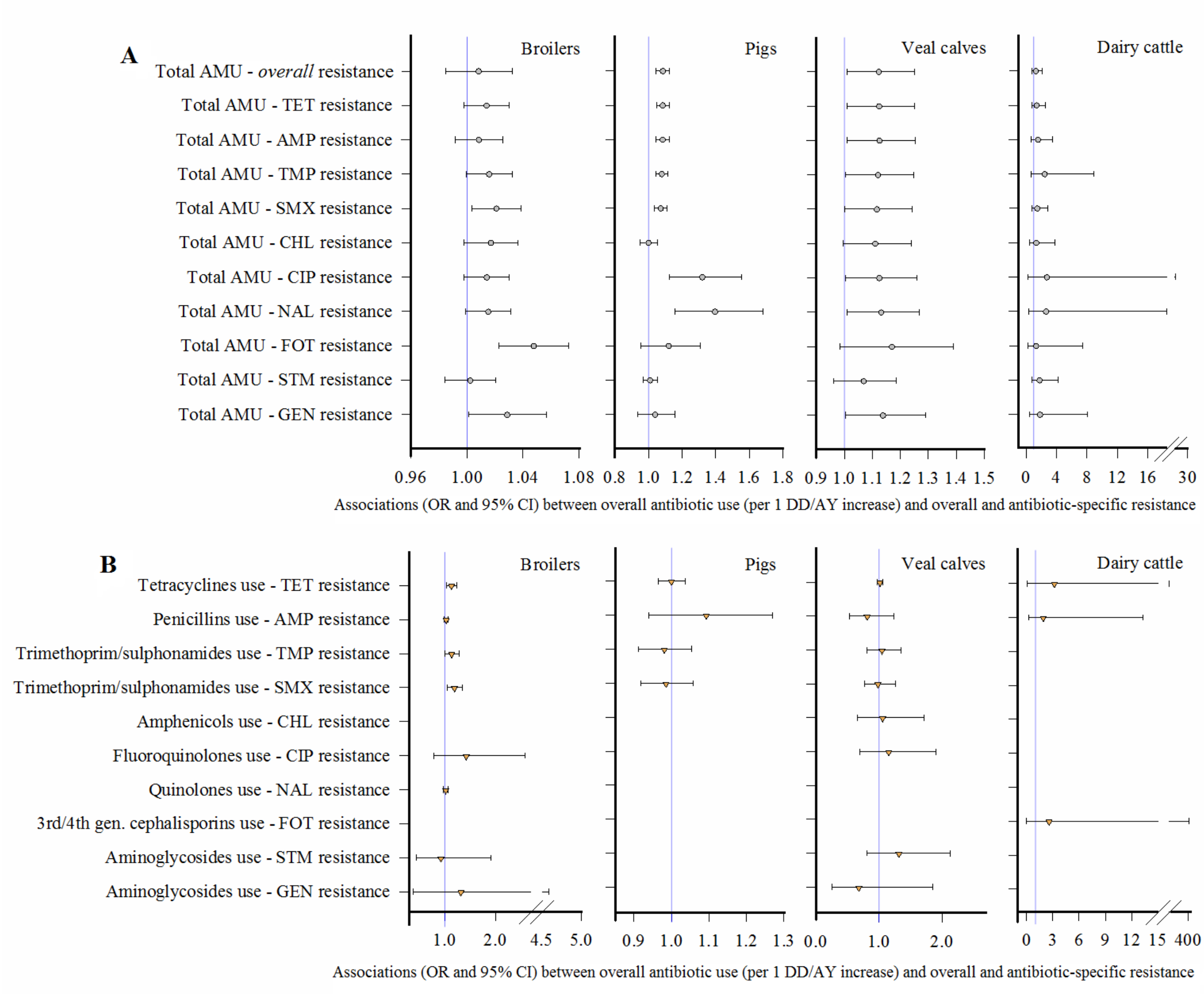
In most livestock sectors, total and antimicrobial-specific usage levels are clearly associated with the antimicrobial-specific resistance levels. This is most prominent in the pig farming sector and less so in the poultry farming sector (see Figure 2). Although not all of these associations are statistically significant, nearly all of them represent a positive trend, with higher usage levels corresponding to a higher prevalence of antimicrobial resistance. For the veal farming sector, the associations are relatively weak. Most of the associations observed for this livestock sector are positive, but not statistically significant. A key confounding variable in this respect is probably the change in sampling method. When the data are adjusted for this confounding variable, no correlations can be observed for the veal farming sector (all odds ratios are close to 1). The least prominent correlations are found for the dairy farming sector, probably as a result of the low absolute usage levels and the small absolute changes in usage levels observed over time. For this livestock sector, none of the classes of antibiotics show a clear association between usage and resistance levels.

Any weak associations observed for the various livestock sectors will in part be due to the fact that national usage data were used rather than data from the actual farms that produced the animals concerned.

Total usage levels were usually more strongly associated with antimicrobial-specific resistance than antimicrobial-specific usage levels. This was probably due to occurrence of co-resistance (several types of resistance in a single micro-organism).

These findings show that antimicrobial usage levels (in defined daily doses for animals) are clearly correlated with the prevalence of antimicrobial resistance. The analyses, as well as the scientific literature, also suggest that an additional reduction in usage levels will further decrease the prevalence of resistant micro-organisms.

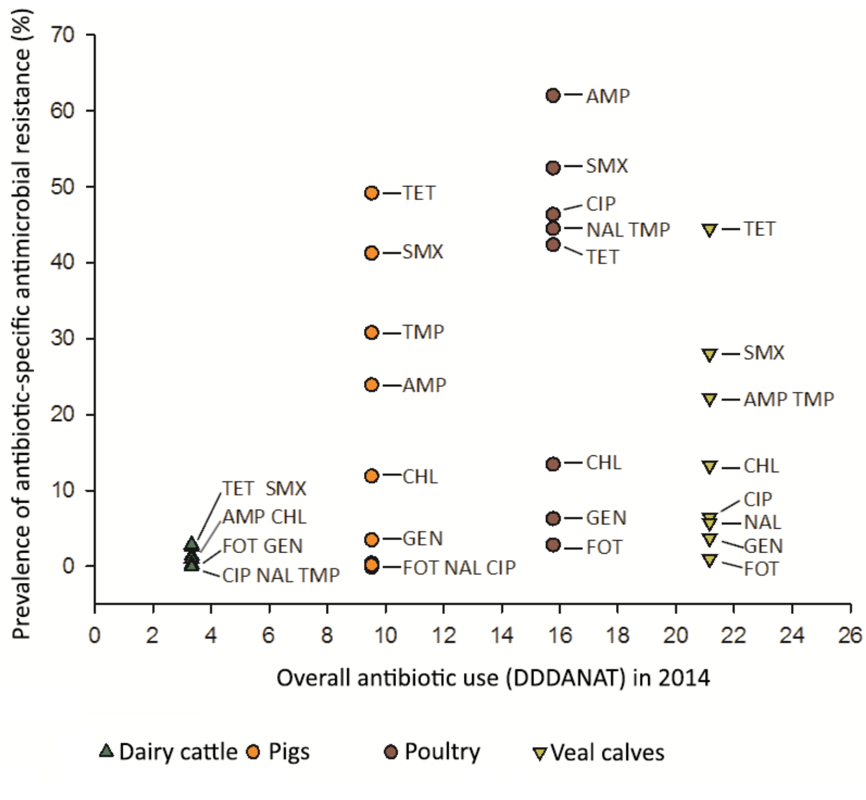
Figure 2. Associations between total antimicrobial use (AMU) and both *overall* and antimicrobial-specific resistance, and associations between class-specific antimicrobial use and antimicrobial-specific resistance. *Overall* resistance is defined as resistance to one or more classes of antibiotics. Associations are expressed as odds ratios. An odds ratio greater than 1 indicates a positive correlation between antimicrobial usage and resistance levels. An odds ratio of 1 indicates that there is no correlation. An odds ratio less than 1 indicates a negative correlation.[7]



The expert panel also wanted to determine the reduction levels necessary to produce similar resistance levels for the three types of juvenile meat-producing animals (veal calves, broilers, pigs). The expert panel therefore decided to rank the livestock sectors based on the total amount of antibiotics used in the most recent monitoring year (2014), and indicate the percentage of resistant isolates for each type of antimicrobial resistance (see Figure 3). The prevalence of resistant isolates turned out to be quite similar for the various livestock sectors. The associations between both total and antimicrobial-specific usage levels and antimicrobial-specific resistance levels lack specificity, in part as a result of multidrug-resistance. It is therefore challenging to translate this information into the actual number of $DDDA_{NAT}$ required for a particular livestock sector to produce similar resistance levels and resistance patterns.

The findings did, however, identify several interesting issues. The prevalence of antimicrobial-specific resistance turned out to vary substantially, from just a tiny proportion to over 50% of isolates for certain types of resistance. In 2014, all livestock sectors recorded low usage levels for fluoroquinolones and third- and fourth-generation cephalosporins, with the broiler and veal farming sectors still recording slightly higher levels (0.2 and 0.02 $DDDA_{NAT}$, respectively).[3] The dairy and pig farming sectors recorded low resistance levels for fluoroquinolones (CIP resistance) and third- and fourth-generation cephalosporins (FOT resistance), while the veal farming sector recorded somewhat higher resistance levels. Resistance patterns in the broiler farming sector were distinctly different, with a low level of resistance to third- and fourth generation cephalosporins (FOT resistance) and a remarkably high resistance level for fluoroquinolones (CIP resistance in >50% of isolates).

Figure 3. Ranking of the four livestock sectors based on total antimicrobial use and antimicrobial-specific resistance in 2014. The results are adapted from previously reported data[4]



Due to the complex associations between total and antimicrobial-specific usage levels and resistance, and the role of multidrug-resistance in this regard, it is not possible to reliably predict how additional reductions in the amounts of antibiotics used will effect resistance levels.

Occurrence of resistance/multidrug-resistance in livestock sectors subjected to monitoring

The table below (Table 3) provides detailed information on the isolates CVI analyzed during the 2007-2013 period. There were no major year-to-year differences in the prevalence of multidrug-resistant micro-organisms. The summary resistance data indicate that mono-resistance (resistance to just one particular type of antibiotic) was relatively uncommon.

Table 3. Detailed information on isolates collected for CVI monitoring (2007-2013)

Livestock sector	# of antibiotics to which resistance was detected	Number of isolates	% of the total number of isolates	% of isolates resistant to the antimicrobial concerned									
				AMP	TET	SMX	TMP	CIP	NAL	CHL	FOT	STM	GEN
Broiler farming sector	0	227	12	0	0	0	0	0	0	0	0	0	0
	1	98	5	37	21	8	1	1	0	0	0	29	3
	2	151	8	32	19	18	3	48	49	1	3	24	4
	3	160	8	44	24	38	24	49	49	5	4	62	3
	4	230	12	82	48	67	50	39	39	7	10	53	6
	5	245	13	82	67	87	74	40	41	13	12	76	8
	6	259	13	84	67	95	80	80	80	22	11	72	8
	7	263	14	94	86	98	92	98	98	18	15	90	13
	8	221	11	99	96	100	91	99	100	80	25	99	11
	9	71	4	100	94	100	99	100	100	90	48	100	69
	10	9	0	100	100	100	100	100	100	100	100	100	100
	Total	1,934	100	67	54	66	55	57	57	21	12	62	10
Pig farming sector	0	406	22	0	0	0	0	0	0	0	0	0	1
	1	216	12	4	62	1	2	0	0	1	0	28	2
	2	218	12	11	65	32	19	0	0	3	0	66	3
	3	247	14	22	84	72	45	1	0	5	0	69	2
	4	269	15	42	77	93	86	0	0	14	3	80	4
	5	344	19	86	98	100	98	1	1	17	1	95	2
	6	88	5	95	99	100	99	8	9	80	6	98	7
	7	14	1	86	100	100	86	86	86	43	21	93	0
	8	12	1	100	100	100	100	100	100	67	33	92	8
	9	0	0	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0	0
	Total	1,814	100	33	63	53	46	2	2	11	2	57	2
Veal farming sector	0	510	38	0	0	0	0	0	0	0	0	0	1
	1	158	12	2	94	1	0	0	0	0	0	3	1
	2	69	5	35	90	9	13	3	3	3	1	39	4
	3	81	6	31	90	58	25	11	11	6	0	68	0
	4	93	7	59	89	88	60	5	5	14	1	76	1
	5	181	14	91	99	97	77	4	4	33	2	91	2
	6	108	8	67	99	99	94	33	34	69	2	94	9
	7	50	4	86	98	100	86	86	78	56	2	88	20
	8	45	3	91	98	98	98	96	93	84	11	100	31
	9	40	3	100	100	100	100	100	100	98	5	100	98
	10	4	0	100	100	100	100	100	100	100	100	100	98
	Total	1,339	100	35	59	42	34	14	14	20	1	41	6
Dairy farming sector	0	1,320	93	0	0	0	0	0	0	0	0	0	1
	1	33	2	9	52	3	0	0	3	3	0	12	18
	2	6	0	33	67	17	0	17	17	17	0	33	0
	3	23	2	43	78	61	13	0	0	9	0	87	9
	4	10	1	70	90	100	50	0	0	0	10	80	0
	5	12	1	83	100	100	83	8	8	25	0	92	0
	6	7	0	57	100	100	71	29	29	43	29	100	43
	7	2	0	100	100	100	100	100	100	0	0	100	0
	8	5	0	100	80	100	80	100	100	80	40	100	20
	9	4	0	100	100	100	100	100	100	100	25	100	75
	10	1	0	100	100	100	100	100	100	100	100	100	100
	Total	1,423	100	3	5	4	2	1	1	1	0	4	1

Types of antimicrobial resistance: AMP ampicillin; TET tetracycline; SMX sulfamethoxazole; TMP trimethoprim; CIP ciprofloxacin; NAL nalidixic acid; CHL chloramphenicol; FOT cefotaxime; STM streptomycin; GEN gentamicin

Most isolates were resistant to several classes of antibiotics. This indicates a state referred to as "co-resistance". In the event of co-resistance, several resistance genes, each of which responsible for a particular type of resistance, are present in the micro-organism concerned. Co-resistance can have major consequences. In the event of co-resistant bacteria, reducing the use of a particular antimicrobial is not guaranteed to lower the prevalence of the gene coding for the corresponding type of resistance.

The highest proportion of susceptible isolates was found in the dairy farming sector (93%). The pig and veal farming sectors recorded substantially lower susceptibility rates (22% and 38%, respectively), and the broiler farming sector recorded the lowest proportion of susceptible isolates (12%). The highest prevalence of multidrug-resistance (resistance to ≥3 classes of antibiotics) was recorded for the broiler farming sector, with multidrug-resistance detected in 75.4% of isolates, followed by the pig farming sector (53.7%), the veal farming sector (45%) and, with a substantially lower prevalence, the dairy farming sector (4.5%).

Resistance patterns turned out to vary distinctly between the various livestock sectors (patterns are indicated by the blue cells in Table 3). Key drivers among multidrug-resistant isolates were ampicillin, tetracycline, sulfamethoxazole, trimethoprim and streptomycin resistance. In the broiler farming sector, ciprofloxacin and nalidixic acid resistance were additional drivers of multidrug-resistance.

Discussion

Interpretation of the analysis results in the light of the scientific literature

The analyses presented in this report revealed associations between levels of antimicrobial use, in terms of both total and antimicrobial-specific use, and the prevalence of specific types of antimicrobial resistance in various livestock sectors. The analyses were performed using ecological data collected in the context of monitoring programs. The data used were not primarily collected for the purpose of such analyses. However, similar analyses using detailed farm-level data on antimicrobial use and antimicrobial resistance have been published before. They include, but are not limited to, the following studies:

- a joint analysis by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Agency (EFSA) and the European Medicines Agency (EMA), comparing the consumption of and resistance to antibiotics in humans and livestock in European countries. For several antibiotics, analysis of 2011 and 2012 data revealed associations between antimicrobial use in livestock and occurrence of resistance in micro-organisms from livestock and humans. For instance, use of cephalosporins and fluoroquinolones was associated with resistance to these antibiotics in micro-organisms from livestock and humans. Positive correlations were also found between use of macrolides in livestock and resistance in human clinical isolates. Associations between usage and resistance were observed for most of the antibiotics included in the analyses. Since the analyses were performed using aggregate country-level data, the results should be interpreted with caution;[5]
- an ecological analysis of antimicrobial use in livestock in 11 countries, using data collected between 2005 and 2008 and taking account of resistance patterns observed in livestock and humans. Strong correlations between usage in livestock and the prevalence of resistance in micro-organisms from humans were observed for several antibiotics, including ampicillin, aminoglycosides and fluoroquinolones. In general, weaker and fewer associations were found when only human consumption and resistant strains in humans were taken into account;[8]
- an ecological analysis of antimicrobial use in cattle, pigs and poultry in seven European countries and the prevalence of antimicrobial-specific resistance in *E. coli* isolates. The groups of antibiotics analyzed were fluoroquinolones and amphenicols, third-generation cephalosporins and sulfonamides, aminopenicillins, fluoroquinolones, streptomycin, and gentamicin and tetracyclines;[9]
- longitudinal studies analyzing antimicrobial use in pigs and veal calves in the Netherlands and the prevalence of livestock-associated MRSA in livestock and humans at individual livestock farms, based on farm-specific isolates (from the livestock farmer, employees and family members).[4, 10] These studies are longitudinal studies rather than ecological studies, and were performed to identify associations between antimicrobial use and resistance for individual livestock farms;

- several similar foreign studies analyzing ceftiofur use and resistance in poultry[11], tetracycline use in pigs and resistant *E. coli* isolates from humans[12], use of various antibiotics and corresponding antimicrobial-specific resistance in pigs at multiple farms[13, 14], and antimicrobial use in veal calves and the prevalence of ESBL resistance[15, 16]. These are just some examples of such studies;
- in most situations, reducing the amounts of antibiotics used results in lower resistance levels, although it may take a while until the maximum effect can be observed;[17, 18]
- several systematic reviews on the associations between usage of and resistance to antibiotics.[19, 20, 21-25]

Considerations regarding the design of published studies

It is worth noting that many of the studies published previously were so called ecological studies, meaning that information is available on an aggregate level only. In epidemiological research, such a design is regarded problematic due to the risk of a so-called "ecological fallacy" resulting in incorrect inference of certain correlations. If the presence of confounding variables differs between the populations that are being compared, ecological correlations (e.g. correlations at a national level) may differ from individual correlations (e.g. farm-level correlations). Epidemiologically speaking, the relationship between usage of antibiotics and the occurrence of antimicrobial resistance is highly complex. It depends on multiple variables, such as the antimicrobials used, the resistance and transmission mechanisms concerned, presence of co-resistant or cross-resistant micro-organisms, and usage patterns. Once a new resistant micro-organism emerges, resistance can spread rapidly, even before the new micro-organism is detected. As a result, an ecological approach is often the only suitable option for detecting and studying resistance. This field therefore relies more heavily on ecological study designs than other fields of epidemiological research. Our knowledge of the emergence and spread of vancomycin-resistant enterococci (VRE), for instance, is largely based on comparisons of data from various countries and continents.

Answers to the research questions and additional observations

1. ***To what extent is the decline in antimicrobial usage levels achieved over the past few years associated with a reduction in the prevalence of antimicrobial-resistant bacteria?***

- The decline in antimicrobial usage levels was associated with reductions in the prevalence of antimicrobial-resistant *E. coli* in fecal samples from veal calves, pigs and broilers. The decrease in prevalence varied widely and depended on the type of resistance concerned. On average, the veal and pig farming sectors showed the strongest decline in resistance levels. For the veal farming sector, a 37.4% reduction in total antimicrobial use was associated with a 26% decline in resistance to one or more classes of antibiotics (*overall* resistance) over the observation period (2009-2014). Since this sector already started to reduce its usage levels in 2007, the total decline over the past few years will exceed the decline reported for the observation period. It should be noted, however, that the veal farming sector's resistance levels are confounded to some extent by a change in the sampling process during the observation period. With 54%, the pig farming sector achieved a more extensive reduction in total usage level. Its overall resistance level, however, decreased by 22%, which was a smaller improvement than the one observed for the veal farming sector.

- The broiler farming sector recorded a smaller decline in antimicrobial resistance (8%), even though total antimicrobial use in this sector decreased by 57%. This might be due to the fact that broilers have a shorter life span than pigs and veal calves (6-8 weeks versus 6-8 months on average). The relatively short period between broilers' final consumption of antibiotics and the time of slaughter might be relevant in this respect. In pigs and veal calves, antibiotics are primarily used at young ages, which generally results in a longer period between final consumption of antibiotics and slaughter. This may mean that at the moment of sampling, the intestinal microbiotas in these animals have had more time to recover from exposure to antibiotics. By then, susceptible strains of micro-organisms have probably largely replaced the resistant strains. Usage of antibiotics earlier in the supply chain might also affect test results.
- Statistically significant correlations were found between the amounts of antibiotics used (total and antimicrobial-specific usage levels) and the prevalence of antimicrobial-specific resistance in *E. coli* isolates.
- In many cases, antimicrobial-specific resistance in *E. coli* isolates was more strongly associated with total usage levels than with antimicrobial-specific usage levels. This might have been due to the presence of co-resistant or cross-resistant micro-organisms.

2. *To what extent does the prevalence of antimicrobial-resistant bacteria vary between the various livestock sectors?*

- Many *E. coli* strains are multidrug-resistant, i.e. resistant to three or more classes of antibiotics. According to this definition, 4.5% of strains isolated from dairy cattle in 2014 were multidrug-resistant. The prevalence of multidrug-resistant strains was quite different in the other livestock sectors, with 45.0% for the veal farming sector, 53.7% for the pig farming sector and 75.4% for the broiler farming sector. The multidrug-resistance rates in these livestock sectors were high, particularly considering the bacteria concerned were isolated from healthy animals. The low prevalence of multidrug-resistance in the dairy farming sector can be explained by this sector's low antimicrobial usage levels and its practice of using selective rather than whole-herd treatment regimens. In this livestock sector, variations over time could be due to the fact that pooled fecal samples (from animals of all ages) were used for some of the tests.
- The broiler farming sector managed to reduce its resistance levels substantially over the past few years, with the prevalence of resistance to third- and fourth-generation cephalosporins showing the biggest improvement. The fact that these antibiotics are no longer used at hatcheries presumably was the main driver of this improvement. The decline in usage of broad-spectrum penicillins as a second-choice agent at broiler farms probably was another contributing factor. A prior policy decision to heavily restrict usage of third-choice agents did not yet markedly decrease the

- prevalence of resistance to quinolones and fluoroquinolones. The proportion of *E. coli* isolates resistant to fluoroquinolones was still high in the broiler farming sector (46.4%).
- The associations between reductions in antimicrobial use and reductions in the prevalence of antimicrobial resistance in *E. coli* isolates observed for each of the livestock sectors, strongly suggest that an additional reduction in usage levels will further decrease the prevalence of resistant micro-organisms.
- However, these associations lack the strength and specificity to facilitate predictions regarding future developments in resistance levels.

3. ***Do the results indicate that associations between antimicrobial use and antimicrobial-resistant bacteria can serve as the basis for benchmarking thresholds?***

- As yet, the associations between antimicrobial use and antimicrobial resistance revealed by the analyses do not indicate a particular usage level below which the level of resistance is equal to the background level (threshold value).
- Resistance thresholds regarding the relationship between use of and resistance to antibiotics could serve as the basis for new benchmarking thresholds. However, current scientific literature does not allow for such threshold values to be derived.
- Furthermore, it is not yet possible to define an "acceptable resistance level". This would require information on resistance-related risks such as public health risks, and currently available data do not allow for quantification of such risks. If an acceptable resistance level were to be determined, the corresponding antimicrobial usage level could serve as the basis for benchmarking values. As yet, however, no acceptable resistance level has been defined.

Conclusions

No benchmarking thresholds indicating an acceptable resistance level can be derived from currently available data. The existing pragmatic benchmarking approach therefore remains crucial for providing insight into the amounts of antibiotics used in the Dutch livestock sector. According to the expert panel, it would also be impossible to determine concrete reduction goals, assuming all livestock sectors would have to strive for similar resistance levels. The associations between antimicrobial-specific and total usage levels and the prevalence of resistance and multidrug-resistance are too complex to do so. The expert panel feels each livestock sector should continue to focus on the livestock farms within the action zone (red) and the signaling zone (orange) in order to further reduce its antimicrobial usage levels in the years to come. This is in line with the recommendations included in the SDa report "Usage of Antibiotics in Agricultural Livestock in the Netherlands in 2014", which was published in September 2015.

With regard to juvenile meat-producing animals in particular (veal calves, broilers, pigs), levels of resistance to several classes of antibiotics are still high and co-resistance and cross-resistance have been observed. The expert panel therefore wants the three livestock sectors concerned to further reduce their usage levels for all classes of antibiotics. This decision is based on the precautionary approach to public health, with consideration of preconditions regarding animal health and animal welfare.
In order to further reduce the amounts of antibiotics used, it is necessary to find out why certain livestock farms have high usage levels. Additional investigation is therefore required to identify determinants of antimicrobial use and to define appropriate interventions. The parties concerned should subsequently implement these interventions and evaluate their effect.

Recommendations

The current Dutch resistance monitoring system is based on EU legislation, and predominantly involves testing of livestock isolates collected at slaughterhouses. The test results give an idea of the effects antimicrobial use has on the prevalence of resistant intestinal bacteria in the various types of livestock. When this information is combined with data on the prevalence of resistance in bacteria isolated from meat, it provides insight into the risk of resistant strains being transferred to humans through direct contact, the environment or the food chain. These data are, however, just an indication of the prevalence of antimicrobial resistance at individual livestock farms. Although the process of testing livestock isolates collected at slaughterhouses can quite accurately determine the risk of resistant bacteria spreading to consumers through the food chain, it does not take account of other relevant forms of transmission. After all, work-related transmission, transmission from people with work-related high exposure levels to their family members or the general population, environmental transmission and transmission within and between individual livestock farms may also be involved. Sampling at slaughter houses also does not give a proper indication of how usage and resistance levels are correlated. The expert panel therefore proposes that regular surveys be conducted to assess resistance-related issues and developments at individual livestock farms. It also recommends refining the monitoring method. It feels it would be helpful to collect antimicrobial usage data from livestock farms that provide fecal samples for antimicrobial resistance testing. The additional information would help determine the relationship between usage and resistance levels, and might at a later date lead to better substantiated benchmarking thresholds. As a final recommendation, the expert panel suggests that results from resistance testing performed at livestock farms in the action zone (red) or signaling zone (orange) should be communicated to individual livestock farmers and the livestock sectors concerned.

Current policy mainly focuses on usage of first-, second- and third-choice agents, with application of third-choice agents being restricted to human medicine as much as possible. The classification of first- and second-choice agents is largely based on the recent emergence of resistant ESBL-producing organisms. Once new types of resistance emerge, this classification may have to be updated accordingly. Even first-choice agents cannot be deemed 100% risk free. The expert panel therefore recommends to continue reducing usage of all antibiotics, first-choice agents included.

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Appendix 1

Participants at the June 2015 expert meeting

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Associations between antimicrobial use and the prevalence of resistant micro-organisms
Is it possible to benchmark livestock farms based on resistance data?
SDa/1147/2016