



Veterinary public health: Human health hazards associated with the administration of antimicrobials to slaughter animals

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HUMAN HEALTH HAZARDS ASSOCIATED WITH THE ADMINISTRATION OF ANTIMICROBIALS TO SLAUGHTER ANIMALS

PART II. AN ASSESSMENT OF THE RISKS OF RESISTANT BACTERIA IN PIGS AND PORK

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SUMMARY

Risks for the consumer regarding the acquisition of resistant bacteria and/or resistance genes via the consumption of pork are discussed. In general, *Salmonella* spp. and *Escherichia coli* that originate from animals do not easily transfer their resistance genes to the resident intestinal flora of humans. The prevalence of resistant *E. coli* in hu-

mans seems more associated with being a vegetarian (odds ratio (OR) 1.89) than with the consumption of meat and meat products. Other risk factors are treatment with antimicrobials (OR 2-5), becoming hospitalized (OR 5.93), or working in a health setting (OR 4.38). In the Netherlands, annually an estimated 45,000 people (0-150,000) become a carrier of resistant *E. coli* and/or resistance genes that ori-

ginate from pigs, while an estimated 345,000 persons (175,000-600,000) become a carrier of resistant *E. coli* and/or resistance genes that originate from hospitals, e.g. other patients. Any problems with resistant *Salmonella* spp. that stem from pigs are, in fact, an integral part of the total problem of food-borne salmonellosis. Sometimes there are outbreaks of a specific multi-resistant clone of *S. typhimurium* that causes problems in both farm animals and humans. The probability that in the next 30 years there is no or maximally one outbreak of a specific clone that originates from pig herds is estimated at about 75%. Antimicrobials used as a growth promoter can have a measurable influence on the prevalence of resistant bacteria. The likely chain of events regarding avoparcin and the selection and dissemination of resistance against vancomycin in the enterococci gives the impression that the impact of the use of antimicrobials in animals on the prevalence of resistance in humans is largely determined by whether resistance genes are, or become, located on a self-transferable transposon. Furthermore, consumer health risks of antimicrobials used in slaughter pigs are mainly determined by the selection and dissemination of bacterial resistance and much less by the toxicological properties of any residues in pork. It is also concluded that most of the problems with resistant bacteria in humans are associated with the medical use of antimicrobials, and that the impact of particularly the veterinary use of antimicrobials is limited. However, the impact of antimicrobials used as a feed additive appears to be much greater than that of antimicrobials used for strictly veterinary purposes. The use of antimicrobials as a feed additive should therefore be seriously reconsidered.

INTRODUCTION

In the Netherlands, a research project called 'A Risk Assessment-Like Approach to the Modernization of Meat Inspection' was initiated to investigate which approach would enable the assessment and comparison of the microbiological and toxicological risks of the production and consumption of meat (19-24). This would make it easier to set objective priorities about which risks should be controlled. For several reasons, the research project was focused on the production and consumption of pork (19-24).

Human health hazards associated with the administration of antimicrobials to slaughter animals can, overall, be grouped into the following three categories: (i) toxic, allergic, mutagenic, teratogenic, or carcinogenic effects of residues in meat and edible by-products (2,5,7,9,11,49,70,125); (ii) disturbance of the normal intestinal flora of individuals by residues (7,9,39,43,49,52,61,78,116,135); (iii) transmission of bacterial resistance against antimicrobials from animals to humans via meat and edible by-products contaminated during slaughtering and dressing (7,14-16,30,31,39-41,49,52,61-64,69-73,78-81,106,110,113). This paper discusses this last aspect with regard to the production and consumption of pork, while the other two hazards are discussed in a companion paper (25).

The approach adopted for risk assessment in this study was based on a highly detailed description of the current knowledge regarding the fate of a particular hazard in the entire pork production chain, from 'stable-to-table', including which factors and circumstances have an influence on the prevalence of the hazard (i.e., a 'descriptive epidemiological model') (8,19,20,25).

An account of the terminology used, how and why different kinds of models can be used, the actual construction of such models, the subsequent analysis of the data included in the different models, the further uses of descriptive models, and the limitations of such approaches is given by Berends *et al.* (20).

The model for resistant bacteria in pigs and pork was primarily built with data from the literature as described in the companion paper (25). Data from the literature were only included in the final model if they were an estimate of a risk or if they could be reprocessed to estimate risks. Some data were obtained from national reports (7,9,10,19,47,66).

In all the risk assessments made, the human health risks were standardized as the (expected) frequency of new cases of disease, contamination, or a clinically apparent pathophysiological effect over a period of 1 year (i.e., the annual incidence). The influence of certain identified risk factors was quantified with the measure Odds Ratio (OR) and occasionally with the measure Attributable Fraction (AF). The strength of the associations was tested for significance with the Chi-Squared Test or, when appropriate, the Fisher Exact Test (12,105). The OR is the ratio between the odds of disease or contamination in the case that the factor is present or absent, and the AF is an estimate of the proportion of cases that is really caused by the factor being present in a particular (sub)population. When the OR of a factor is significantly larger than 1 it is a definite risk, and when it is smaller than 1 it works preventively.

AN ASSESSMENT OF HUMAN HEALTH RISKS RELATED TO RESISTANT BACTERIA IN PORK AND EDIBLE BY-PRODUCTS

1. Hazard identification:

The use of antibiotics in slaughter animals for the prevention and treatment of disease as well as for growth promotion is believed to contribute significantly to the problems with infections with resistant pathogens in humans. Pork is incriminated as a relatively important source of (i) resistant zoonotic bacteria that are often carried by slaughter animals, such as *Salmonella* spp., and (ii) bacteria that are mostly non-pathogenic and/or cause opportunistic infections, but that can transfer their resistance genes to other bacteria, such as *E. coli* and the enterococci (1,14-16,26,31,39-41,47,49,52,59,61-64,69-73,78-81,85,87,97,106,110,113,116,125,132,145,155). With regard to the enterococci, not only the simultaneous resistance against ampicillin, gentamicin, and vancomycin is feared, but also the possibility that at some time in the future vancomycin-resistance will be transferred from the vancomycin-resistant enterococci (VRE) to the methicillin-resistant *Staphylococcus aureus* (MRSA). This would make an infection with the latter agent virtually untreatable.

2. Dose-response characterization:

From the literature, it was calculated that the OR of antibiotic treatment as a risk factor for a subsequent isolation of resistant bacteria from human or animal faeces, pus, blood or urine, generally varies between 2 and 5, but has been calculated as high as 10 (4,21,33,44,52,59,63,74,81,86,101,114,115,119,

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Table 1. Effects of antimicrobials on the prevalence of resistant bacteria, transmission of resistance genes, or extra-intestinal location of resistant bacteria (prospective cohort studies).

Factor	Effect	Data	OR & RR	95% CI	Ref
Therapeutic doses of broad-spectrum antibiotics in gnotobiotic animals colonized with <i>E. coli</i> only	Transmission of resistance genes from <i>E. coli</i> to fully sensitive <i>Salmonella</i> spp. in the intestinal tract	y +61 n -42	OR: 7.68 RR: 2.25	3.65-16.36 1.72-2.93	144
see above	Resistant <i>Salmonella</i> spp. in organs	y +64 n -25	OR: 3.60 RR: 2.84	2.11-6.17 1.86-4.34	144
80 mg/kg kanamycin in the feed	Transmission of resistance genes from <i>E. coli</i> to <i>Salmonella</i> spp. in the intestinal tract	y +23 n -4	OR: 115 RR: 5.75	11.82-1119.10 2.34-14.14	56
80 mg/kg virginiamycin in the feed	50%-100% of the total number of enterococci in the faeces resistant to erythromycin	y +47 n -19	OR: 7.80 RR: 2.47	3.40-22.47 1.67-3.67	65
>10 mg/kg avoparcin in the feed	Colonization of the intestinal tract with multi-resistant gram-negative bacteria	y +55 n -11	OR: 225.00 RR: 5.00	27.89-1815.46 2.94-8.51	161

OR = odds ratio; RR = relative risk; 95% CI = 95% confidence interval; Ref = reference on which the calculation was based; + factor present, - factor absent; y effect present, n effect absent

124,128,129,134,140,148,162). The increase in the prevalence of resistant bacteria in a particular host is the result of selection and/or of a contamination from outside sources. Bacteria can acquire resistance by spontaneous mutation or via resistance genes that are transmitted between different strains and species. In addition, it can be inferred that between 80-90% of all problems with infections with resistant bacteria are caused by transmission of resistance genes via plasmids and transposons (see also below) (43,45,58,62,68,72,76,92,99,104,113,120,131,139,147,159,160).

An American study of three different pig herds provides some insight into the effects of antimicrobials used as growth promoter. In this study (i) one pig herd was never exposed to antimicrobials (herd A) (46); (ii) one pig herd was only treated for disease (herd B) (59); and (iii) one pig herd was also fed some antimicrobials at very low doses as a growth promoter (herd C) (83). Calculations based on the published data showed that in herd C there were on average 4.1 times more *E. coli* strains resistant to one or more antimicrobials than there were in herd A, while in herd B there were on average 3.4 times more resistant isolates than there were in herd A. The differences between B and C were consistent and statistically significant (χ^2 : 29.4; 10 df; $P < .01$). Looking specifically at those antimicrobials that were used as a growth promoter, the correlation between this purpose and the extra increase in the percentage of resistant strains was high (Pearson's coefficient: 0.89; $P < .05$). When compared with herd B, the additional use in herd C of Beta-lactam antibiotics, tetracyclines and aminoglycosides as growth promoter resulted in a 2.1-, 1.4- and 2.8-times higher prevalence of *E. coli* resistant to these substances, respectively. In herd C, the prevalence of resistance was on average 1.2-times higher. In other words, on average 20% (0-65%) of the bacterial resistance in herd C may have been caused by the concomitant use

of another, mostly chemically unrelated, antimicrobial as a growth promoter (i.e., the attributable fraction, AF). Furthermore, the *E. coli* isolated from herd C were resistant to on average 3.7 different antimicrobials and those from herd A to on average 1.8. This phenomenon is also called co-selection, which is a common consequence of the use of antibiotics (27,31,68,69,81,92,101,150,159).

A normal intestinal flora appears to have, among other things, a significant influence on: (i) the measure in which ingested bacteria, yeasts, and fungi are being prevented from residing in the intestine and/or causing disease; and (ii) the frequency with which conjugation and subsequent transmission of resistance genes on plasmids takes place within and between the bacterial species present in the intestinal lumen (29,135-137,152,154). The bacteria that influence this are called the colonization-resistant flora, of which the Gram-positive obligate anaerobic species are the most important (136,137).

Though many factors are supposed to affect the composition of the colonization-resistant flora, such as age or stress, broad-spectrum antimicrobials have the most influence (21,29,32,69,136,137,154). The effects on the flora of a single oral therapeutic dose of broad spectrum antimicrobials with an effect on Gram-positive bacteria can be observed for about 24-48 hours, and the effects of repeated doses for about 7 days (21,29,32,39). A short exposure to a daily amount of antimicrobials less than 5-10% of the normal therapeutic dose is seemingly without effect on the composition of the intestinal flora of adults and/or the prevalence of resistant bacteria (25). However, continuous exposure via food or animal feeds that contain more than 5 mg/kg (wet weight) does have an influence on the prevalence of resistance (7,25,43,46,56,57,65,71,83,87,161).

Although the numbers of *Salmonella* spp., *E. coli*, and

Table 2. Risk factors regarding the isolation of resistant *E. coli* from humans (cross-sectional studies).

Risk factors	Resistant <i>E. coli</i> ?		OR	95% CI	AF	Ref
	Yes	No				
Vegetarian vs. Non-vegetarian	16 26	12 25	1.3	0.54-3.25	-	6
Vegetarian vs. Non-vegetarian	28 20	49 66	1.9	0.90-3.95	-	101
Vegetarian vs. Non-vegetarian	110 54	340 316	1.9	1.30-2.76	47%	52
<3 months after therapy with antibiotics v.s. No therapy	50 97	10 53	3.1	1.37-7.27	67%	109
<3 months after therapy with antibiotics* v.s. No therapy	4 8	70 641	4.6	1.34-15.62	77%	52
Hospitalized adult (but not treated with antibiotics) vs. a member of the general population (adult)	74 26	12 25	5.9	2.61-13.49	62%	6
Nurse vs. a member of the general population (adult)	82 25	18 25	4.4	2.07-9.28	59%	6

OR = odds ratio; RR = relative risk; 95% CI = 95% confidence interval; AF = attributable fraction; ref. = reference on which the calculation was based. *infection of the urine tract

enterococci required to colonize the human intestine are highly variable, and minimal or average required doses are not known, it can be calculated that a therapeutic dose of broad-spectrum antimicrobials reduces the number of bacteria needed by a factor 100 to 10,000 (19,21,29,32,63,152, 158). Therapy with broad-spectrum antibiotics has, as a risk factor for a subsequent colonization of the intestinal tract with *Salmonella* spp., an Odds Ratio (OR) of on average 5.5 (21,86,119,124). Furthermore, from a cohort study it was calculated that the OR of a therapeutic dose of tetracyclines as a risk factor for 'being colonized for more than 5 days with an exogenous resistant *E. coli* strain' is 21.11 (95% confidence interval (CI): 2.82-206.37), and that the relative risk (RR) is 5.6 (95% CI: 1.89-16.83) (39).

Conjugation and subsequent transmission of resistance genes on plasmids between *Enterobacteriaceae* or between enterococci are assumed to be negatively influenced by the anaerobic conditions in the intestinal lumen, the presence of free volatile fatty acids, such as propionic and butyric acid, and the differences in numbers between these species and the obligate anaerobic Gram-positive species (45,61,62,72,76,93,144). Most research shows that, for a detectable *in vivo* transmission of resistance genes the composition of the intestinal flora has to be out of balance. This occurs in newly hatched chicks that have no gut flora, in gnotobiotic animals colonized with *E. coli* only, or in animals continuously administered both donor and recipient strains. In these experiments, the donor and recipient strains were always present at levels up to 10^8 - 10^9 colony forming units (cfu) per gram faeces, while the ratio between donors and recipients varied between at least 2:1 to 1000:1 (39, 43, 62, 63, 76, 78, 81, 144). Even then, the success rates varied between 20-100%, and only additional use of antibiotics ensured 100% success (56,83,90). In conventionally raised animals, a transmission of resistance genes can only be detected if the intestinal flora is disrupted beforehand, i.e. by antibiotics or as a result of massive diarrhoea (39,57,63,76,78,144). When this was the case, a transmission of resistance genes was ob-

served in 10-20% of the individuals (62,114). In addition, from the data of Kuske-Nijsten (81) it can be inferred that when the intestinal flora of an experimental animal is not disturbed, the *in vivo* transmission frequency of resistance genes is a factor 100 to 100,000 lower than the *in vitro* frequency, and that when the intestinal flora is disturbed the *in vivo* transmission frequency of resistance genes are almost as high as the *in vitro* frequencies. Table 1 lists the influence of antibiotics as a risk factor on the transmission of resistance genes in the intestinal lumen and/or recovery of bacteria with these particular resistance genes.

However, it is not known in how many subjects resistance genes acquired from exogenous *E. coli* persist in the intestinal flora without the selective pressure of antibiotics (29,39,52, 92,101,144). It seems that transconjugants do not persist for very long at detectable levels without some kind of selective pressure. An experiment with human volunteers showed that when *E. coli* were administered in doses of up to 10^9 cfu, only 4 of 18 different *E. coli* strains from animal and human origin could be recovered from stools (78). One pig strain could be isolated for about 5 days, 1 cattle strain for about 18 days, and 2 human strains for 7 and 35 days, respectively. Transmission of the characteristic resistance patterns to the *E. coli* biotypes that were resident flora was detected 3 times, and these 'transconjugant' *E. coli* could be isolated for 2, 18 and 35 days, respectively. In another study, 8 of 24 people who were given doses of 10^6 cfu *E. coli* of human origin became colonized for on average 4 ± 2 days, while 9 of 10 people that were given therapeutic doses tetracyclines became colonized for 9 ± 3 days (39). Transmission of the characteristic resistance trait was not detected. Remarkably similar effects of broad-spectrum antibiotics were observed in a colonization experiment with gnotobiotic rats and vancomycin-resistant enterococci (i.e., colonization for on average 9 days with and for 4 days without simultaneous administration of antimicrobials) (158). Lastly, a follow-up study regarding the persistence of a particular hospital-acquired, unique, resistance gene showed that

Table 3. Estimates of the probability of colonization with exogenous *E. coli* or *Salmonella* spp. of human or animal origin.

Number of samples	% Colonized (and 95% CI)	Details	Period	Ref
<i>In the hospital, with fellow patients as the source</i>				
100	6% (2-13)	Normal wards	2-3 days	44
50	23% (12-36)	Normal wards	3 weeks	44
63	33% (23-48)	Childrens' wards	2 weeks	109
147	7% (3-12)	Intensive care units	1 day	142
--	57% (49-65)	Nursing homes	6 months	19
76	70% (60-82)	Nursing homes, secondary human-to- human spread of initially foodborne salmonellosis	3 weeks	19
<i>At home, with family members or food of animal origin as the source</i>				
26	4% (0-20)	Secondary spread of a particular <i>E. coli</i> in families of people recently discharged from the hospital	6 months	142
264	7% (4-11)	Human-to-human spread of initially food-borne salmonellosis in families	1-7 days	19
15	7% (0-30)	Household preparation of chickens that were purposely contaminated with a particular <i>E. coli</i> strain	-	52
922	0.22% (0-0.5)	<i>E. coli</i> in human stool samples with a resistance pattern that was unique for pigs in the same region	3 years	4
400	0.25% (0-0.6)	<i>E. coli</i> in human stool samples with a resistance pattern that was unique for pigs in the same region	-	81
<i>At work, with animals as the source</i>				
259	0.39% (0-2.5)	Colonization of pig farmers with <i>E. coli</i> with their animals as the most likely source	-	81
649	0.15%~ (0-1)	Infection of slaughterers with an <i>E. coli</i> of animal origin, with ~ and without * having been treated with antibiotics	3 months	52
74	4.2%* (1-11)	Colonization of slaughterers with <i>Salmonella</i> spp.	3 months	20
-	0.50%			

95% CI = 95% confidence interval; Ref = reference on which the calculation was based;

- = not applicable.

the resistance could not be detected in 12 of 25 carriers (48%) within 3 weeks after their discharge from the hospital. When the remaining 13 carriers were sampled again after 5 months, only 2 of them still carried detectable levels of *E. coli* that possessed the unique resistance gene (8%) (142).

3. Human exposure characterization:

3.1. Exposure to antibiotics:

Based on several sources, it was estimated that in the Netherlands annually between 6-10% of the population receive antibiotics (5,10,38,42,52,81,94,101,115). Between 2-4% of the population receive antimicrobials after they are hospitalized (20-30% of the hospitalized are treated with antimicrobials), and about 4 to 6% are prescribed antibiotics by their general practitioners. Furthermore, farmers, veterinarians, and slaughterers are significantly more often medicated with antimicrobials than the general population, and these three groups also differ significantly from each other (19,24,33,81,101).

The penicillins and tetracyclines are the most frequently used substances in both human and veterinary medicine (25,33,42,107). Vancomycin and third-generation cephalosporines are used sparingly in Europe. In Denmark, for example, the total nationwide use of vancomycin amounts to approximately 20 kilograms per year (47,79).

3.2. Exposure to resistant bacteria:

Salmonella spp.

It is estimated that more than 95% of all human infections with *Salmonella* spp. in the Netherlands are food-borne (20-24,67,71,114). About 15% (5-25%) of these cases is associated with pork, whereby between 5 and 30% of retail-ready pork and 10 to 60% of minced pork and sausages is contaminated (23,24).

Over the past 30 years, there have been four outbreaks of human salmonellosis with specific multi-resistant *Salmonella typhimurium* clones that also caused some disease in food animals in the Netherlands. Three times the clone was clearly associated with British calves (*S. typhimurium* phage type D204, D193, and DT104) and one time with Dutch pigs (*S. typhimurium* phage type 505) (64,87-90, 96,110,127). In none of these outbreaks were the characteristic resistance patterns transmitted to other *Salmonella* spp. or other *Enterobacteriaceae*. Furthermore, slightly less than 5% of all *Salmonella* spp. isolated from humans in the Netherlands is multi-resistant (64,87-89).

Escherichia coli

The situation regarding *E. coli* is different from that of the *Salmonella* spp. It is undoubtedly true that carcasses become contaminated with *E. coli* strains from animal origin during

slaughtering (78,109,155), and it has been calculated that under routine conditions 25 to 50% of Dutch pork carcasses may become contaminated with on average 100 cfu Enterobacteriaceae/cm² (modus 1.6 log₁₀; median 3.2 log₁₀), and that the remainder may become contaminated with numbers below the detection limit of 20 cfu/cm² (19, 22, 60). However, after cooling, storage, cutting, distribution and further processing into retail-ready products, 90-99% of the coliform organisms present are not of animal origin (22,78,82,112,126,155).

Furthermore, despite close contacts with animals, the *E. coli* flora of pig farmers is phenotypically rather homogenous, whereas their animals have phenotypically a very clear relationship with the type of farm where they are kept as well as with the farm-specific use of antibiotics (81). Table 2 shows that the presence of resistant *E. coli* in human faeces is clearly associated with personal use of antibiotics, becoming hospitalized, working in a health setting, or being a vegetarian. Meat is seldomly consumed uncooked, whereas more than 50% of raw vegetables and salads contain multi-resistant Enterobacteriaceae at levels of 10² to 10⁴ cfu/gram (93). Fruit, vegetables, and salads that are consumed raw seem, therefore, a more likely source of resistant *E. coli* than normally prepared meats (52). Table 3 lists the estimates that were made on the basis of data in the literature. It expresses the probability that a person becomes contaminated with a resistant *E. coli* of human or animal origin in a given epidemiological setting. It seems that humans are a more important potential source than animals. The hospital, in particular, is an epidemiological setting that offers many opportunities for direct and indirect cross-contamination coupled with a high selective pressure by antibiotics. Pig farmers seem to have a 20 times, and slaughterers a 7 times higher risk of becoming colonized with an *E. coli* from animals than members of the general population. This agrees with estimates made about the higher risks of a *Salmonella* spp. infection incurred by farmers and slaughterers (20,24).

Furthermore, carbadox, apramycin and nourseotrichin are antimicrobials that are used exclusively in animals in Europe. Nourseotrichin has never been used in the Netherlands. In the Netherlands, 0 of 110 (0%) sampled veterinarians carried *E. coli* with carbadox resistance (33). Another Dutch study found that 1.1% of farmers (3/278), 0.3% (1/289) of slaughterers and 0.6% (1/180) of members of the public carried *E. coli* with apramycin resistance (i.e., AAC(3')-IV aminoglycoside resistance) (81,101). The data regarding the use of nourseotrichin in the former German Democratic Republic did not allow an estimate to be made of the prevalence in farmers, slaughterers, and the public (149). The study did show, however, that resistance genes that were located on a self-transferable transposon became prevalent in *E. coli* strains from members of the public after nourseotrichin had been widely used as a growth promoter in pigs for about 2 years, and that at pig farms resistance had also been transmitted to other members of the Enterobacteriaceae.

Furthermore, in pig houses, calf stables and in hospital wards, it has been observed that massive spread of resistance genes mostly takes place outside the intestinal lumen, and often under antibiotic pressure (97,98,107,142). In hospitals, the skin, the bladder, urine catheters and urine collection bags were more likely places for conjugation and subsequent transmission of plasmids and resistance genes than the intestinal lumen itself. In pig houses and calf stables it was the faeces-infested environment and the continuous faecal-oral cycle that played a decisive role.

Lastly, it can be concluded that *Klebsiella*, *Proteus* and *Pseudomonas* spp. play a much more important role than *E. coli* in the epidemiology of human infections with resistant bacteria (13,58,95,100,102,103,107,131,133, 157,160,163). This is partly because *Proteus* and *Klebsiella* spp., in particular, are in daily practice better and more active donors and recipients of plasmids than *E. coli*, partly because they survive better in the environment, and partly because they are more pathogenic than *E. coli* (37,102,121,142).

Vancomycin-resistant enterococci (VRE)

The problems with resistance against vancomycin located on a self-transferable transposon are, until now, mainly caused by *Enterococcus faecium* and, to a lesser extent, *E. faecalis*, two species that are normal inhabitants of the intestine of many animals and humans (1,28,66,73,77,108,134,145). The enterococci are opportunistic organisms that normally do not cause infections. In seriously debilitated patients, however, they can become life-threatening. Furthermore, until recently vancomycin was the last option in case of resistance to ampicillin and gentamicin. Enterococci play a role in 10-15% of nosocomial infections, with, when untreated, a mortality of up to 40-60% (28,50,75,99,104,117,120,122,134,147,148). In the Netherlands, 3 to 6% of patients acquire a nosocomial infection and up to 1% of the enterococcal infections are caused by VRE, though these infections are still treatable since these VRE are, as yet, not simultaneously resistant to ampicillin and gentamicin (1,53,84,162).

Furthermore, enterococci are normally excreted in large numbers (10⁹ cfu/gram faeces), and survive for a long time on many kinds of surfaces. The proportion of people who become colonized via direct and indirect contacts with carriers is comparable to that calculated for the Enterobacteriaceae (table 3), although, in contrast to most of the Enterobacteriaceae, these enterococci are known to persist for a year or longer in the gastrointestinal tract of humans (34,35,108,111,136,137,141, 154).

Recently, it has become clear that the use of the vancomycin related antimicrobial avoparcin as a growth promoter has led to VRE being present in 40-60% of pigs and poultry in several countries in Europe (14-16,18,31,36,40,48,73,80,106,138, 156,164). The VRE have also been isolated from carcasses and meat, with up to 15% of the retail-ready pork being positive with less than 100 cfu VRE/gram, and from the environment, i.e., sewage and rivers (15,16,18,40,48,138,156). In the Dutch province of Limburg, 50% of turkeys, 39% of turkey farmers, 20% of turkey slaughterers, and 13% of members of the public carried VRE (30). In the city of Rotterdam up to 3% of the public, 48% of pet dogs and 16% of pet cats carried VRE (18). However, two other recent studies in the Netherlands did not clearly establish a relation between the consumption of meat and the colonization with VRE. The first study found 6/62 carriers of VRE in a home for elderly people and 0/42 carriers in a home for the elderly with a strictly vegetarian kitchen (138). This difference was not significant. There is also the question of whether the research was biased, since massive direct and indirect person-to-person transmission of enteric bacteria is usual for institutional settings like these (35,120,141,142, 148). Furthermore, research in hospitals and in the general population has shown that direct and indirect human-to-human transmission of VRE is considerable (15,28,35,54,106, 117,120,134,140,141,148,158). The second study was carried out in the general population, and described 1 carrier of VRE among 276 meat eaters (0.4%) and 0 carriers of VRE among

318 vegetarians (36). Again, this difference was not significant. Also, research in Poland and The United Kingdom indicated that most of the enterococci found in meat and meat products at the retail level is the result of cross-contamination and post-process contamination, respectively, with the environment and the staff as the most likely important sources for, in particular, post-process contamination (40,64).

In conclusion, it can be said that currently: (i) several cycles of contamination and recontamination with VRE exist between animals, humans, and the environment; (ii) the use of certain antimicrobials, such as avoparcin and bacitracin, has played an important role in sustaining these cycles; and (iii) not enough data exist to reveal all the important pathways via which VRE is exchanged within and between the human and animal populations (1,15-17,26,38,40,55,73,77,84,85,143,145,148,151,156,164). It might be that there is a relatively small but steady transmission to the human population via pork and chicken, but that direct and indirect human-to-human transmission, and possibly the considerable output to the environment by humans via sewage, leads to a further amplification of the ongoing contamination cycles with VRE.

4. Risk characterization:

Salmonella spp.

Without a categorization of special risk groups, such as young children, the annual risk for an 'average' consumer in The Netherlands of becoming infected via pork is 6.7×10^{-4} (19,24). The risk of becoming infected via pork with a strain that is multi-resistant is about 5% of that, i.e. 3.4×10^{-5} . This means that, with a consumer population of about 15 million people, annually on average 450 persons become infected via pork with a multi-resistant *Salmonella* sp.

When the infection is uncomplicated, it does not require therapy with antibiotics (41,71,86,128,129,130,132,139). However, on average 5% of cases becomes complicated, so that annually this multi-resistance might become a problem in about 25 people. Problems may arise when therapy with antimicrobials is not only started blindly, but also maintained blindly. If the bacteria in question happen to be resistant to the antimicrobial used, treatment in this manner can often result in worsening of the condition or even in the death of the patient, and might therefore be considered as medical malpractice (3,27,86,114,124,129).

The earlier mentioned four outbreaks over the past 30 years with the food-animals-associated clones of *Salmonella typhimurium* are usually modelled according to a Poisson distribution (152). The probability of 0, 1, 2, 3, 4, or 5 outbreaks with a pig husbandry-associated clone in the next period of 30 years can in this manner be estimated at 37%, 37%, 18%, 6%, 1.5%, and 0.5%, respectively. This means that the probability that this does not happen or happens only once can be estimated to be nearly 75%.

Escherichia coli

The fact that farmers, slaughterers, and veterinarians carry resistant *E. coli* more often than members of the public (81,101), can largely be explained by their significantly higher intake of antibiotics due to professional diseases (33,51,52). Table 3 shows, among others, that people who are hospitalized seem to have a 25 times greater chance of becoming colonized with an *E. coli* from their fellow patients than a farmer has of becoming colonized by a strain from his pigs. Annually, an estimated 2% of pig farmers (1-3%), 0.6% of the personnel at slaughter lines (0-2%), and 0.3% (0-0.6%) of members of the

public become colonized by an *E. coli* strain that originates from pigs. This means that annually about 45,000 people (0-150,000) become colonized for about a week with (mostly) resistant *E. coli* from pigs. It may be that about 10% (4,500 people) become colonized for a longer period. When the hosts' colonization-resistant flora is disturbed, by antibiotic therapy in particular, this temporary colonization will also result in successful transmission of resistance genes to the resident flora in 10-20% of cases. Considering that annually about 4% of the general population is prescribed antibiotics, it may thus be the case that transconjugant *E. coli* with pig husbandry-associated resistance genes can be found, for a period of about a week, in about 300 people (0-1200).

It can also be estimated that annually on average 2% (1-4%) of 15 million consumers become colonized by a hospital-derived resistant *E. coli*, either because they themselves were hospitalized, or because a family member was hospitalized. This means that annually on average 345,000 people (175,000-600,000) become colonized by a resistant *E. coli* that is hospital derived. Of these, about 34,500 may become colonized for several months. Since 20-30% of hospitalized people are treated with antibiotics, the transmission of plasmids and resistance genes from the exogenous *E. coli* to resident strains may succeed in about 13,000 people (3500-36,000).

Thus, it appears that at least 8 times more people are colonized with resistant *E. coli* that originate from other patients in hospitals and/or the hospital environment (i.e., a 'house-flora') than are colonized via pork with resistant *E. coli* that originate from pig farms. Furthermore, successful transmission of plasmids and resistance genes to the resident flora may happen about 48 times more often with 'hospital-derived' resistance genes, in particular, than with pig-farm derived resistance genes. In other words, between 90-98% of the colonization of persons by *E. coli* and/or the transmission of resistance genes to the resident flora are connected with the medical use of antibiotics, and the remainder with the use of antibiotics in animals. This is considered separately from the fact that most problems with the emergence and spread of resistance in hospitals, in particular, are not caused by *E. coli* but by other *Enterobacteriaceae*, of which humans are also the main reservoir.

VRE

The lack of comprehensive data makes it impossible to assess the risks for the consumer of colonization by VRE originating from pigs or poultry accurately. From the exposure characterization, it can be inferred that in Dutch hospitals the current annual incidence of VRE is about 1 in 10,000 enlisted patients, i.e., an annual total of on average 150 patients. However, since these VRE are, as yet, not simultaneously resistant to ampicillin and gentamicin, the infections are still treatable. If this were not the case, 60-80 of these patients would probably die (53,106,117,140,148). It was also not possible to quantify properly what would happen, in terms of human health, if VRE were to transmit its vancomycin resistance to methicillin-resistant *Staphylococcus aureus* (MRSA).

DISCUSSION AND CONCLUSIONS

It is obvious that many aspects of the use of antibiotics in farm animals as well as of the consequences for human health are still largely unknown. For example, there are no hard data about the importance of all possible direct and indirect routes via which animals and humans exchange their resistant bacteria. Reliable data about the probabilities that people or animals become colonized by certain ingested bacteria and the *in vivo*

transmission of resistance genes are also lacking. If a future system of safety assurance of meat is to be based on risk assessment, systematic monitoring of the prevalence of resistant bacteria is necessary. For discovering ongoing contamination cycles, it may be necessary to sample not only animals, humans, meat, and meat products, but also surface water, sewage, vegetables and fruits. Monitoring the quantities of antimicrobials used in animal and human populations is also necessary.

The impact of the veterinary and agricultural use of antimicrobials on the epidemiology of infections with resistant bacteria in humans appears to be determined by whether the bacterial species in question is both able -or enabled- to circulate between animal and human populations, and whether the resistance genes are easily passed on to the resident flora of a newly colonized host. With respect to the potential for transmission of resistance genes to (i) other bacteria, (ii) other bacterial species, and (iii) different populations of eukaryotic hosts, it seems especially important whether a resistance gene is on a self-transferable transposon. The likely chain of events in the selection and spread of resistance against nourseotrichin and vancomycin shows that when a resistance gene is on such a transposon, the penetration of new populations of both bacterial and eukaryotic hosts may be rather disproportional to the original amounts of bacteria or resistance genes transmitted (14,37,45,53,72,76,80,92-94,113,115,142,149,157). Location on a self-transferable transposon seems, therefore, a key factor in a truly successful transmission of resistance genes from animal to human populations. It may be that continuous exposure to low doses of antimicrobials in feed creates just the set of circumstances which makes it highly likely that at some time a resistance gene becomes part of a self-transferable transposon. This scenario is more likely than the short-term exposure to high doses of antibiotics through therapy or preventive veterinary use. It appears that a majority of cases of a highly successful penetration of human populations by animal husbandry-associated resistance genes, in particular, are in some way also associated with the use of antimicrobials as a feed additive and/or with being on a self-transferable transposon (1,14-16,30,31,48,65,71,80,87,106,129,149,157).

The risks of multi-resistant *Salmonella* spp. in pork are small, and they should not be separated from any problems with food-borne salmonellosis in general. More than 95% of cases of salmonellosis in the Netherlands, including the officially recorded 20-30 deaths per annum, are caused by strains resistant to 0-3 antimicrobials (19,64,87-89,110). Furthermore, as shown in practice, the transfer of resistance genes from *Salmonella* spp. to the resident flora of consumers is not likely to occur or to be very persistent without simultaneous selective pressure by antimicrobials (19,56,64,87-89,110,144).

Regarding the risks of a pig-derived *E. coli*, it has been calculated that 90-98% of potential problems in humans with resistant strains of *E. coli* are caused by the medical use of antibiotics. The calculations also show that the human population itself is the main reservoir of these strains as well as of the resistance genes involved. Other studies point in the same direction (91).

Remarkably, the enterococci are especially prevalent in humans, domesticated animals, and wild animals and birds that come into close contact with humans and human waste, such as seagulls near sewage plants and garbage dump sites, whereas enterococci are practically absent from wild animals and birds that have seldomly contact with humans and human society (108). Although the exact routes of transmission are still unclear, this does point out that enterococci circulate between hu-

man and certain animal populations. Nevertheless, based on data in the literature, 90 to 99% of multiple resistant *Enterococcus faecium*, *Enterococcus faecalis* and VRE found in sewage and surface waters actually stem from the human population, even if the surface waters run through mainly agricultural areas (15,17,26,38,55,124,143,151).

For the average consumer it is not very important whether or not the enterococci in his/her intestine are VRE. It becomes so when these consumers get an ailment that predisposes them for an enterococcal infection, especially when they are also treated with antibiotics. This category of people, however, is very small. Furthermore, in Europe, where the prevalence of VRE in humans is high and the medical use of vancomycin is restricted, the incidence of VRE infections in hospitals is at least 10 times lower than in the United States of America, where the prevalence of VRE in humans and animals is low and the medical use of vancomycin is high (and avoparcin has never been used as a growth promoter) (1,50,54,75,80,84,85,99,104,117,118,120,122,123,128,134,140,148).

When compared with the risks of colonization and infection with *Salmonella* spp. that stem from pork (24), the risks for the random consumer imposed by pork with resistant *E. coli* seem to be a factor 10-1000 lower. Furthermore, the consumer risks imposed by resistant bacteria that are selected by the use of antibiotics in food animals appear to be much more important than the current health risks imposed by residues of antimicrobials in pork, which were estimated to be in the order of 10^{-8} (25). In conclusion, the consumer risks associated with the use of antimicrobials in slaughter pigs are primarily due to the selection and dissemination of resistance and not to the toxicological and carcinogenic properties of the substances used. Furthermore, these risks appear to be much smaller than the risks imposed by food-borne infections as such. Clearly, most problems in humans with resistant bacteria are associated with the medical use of antimicrobials. The impact of the veterinary use of antimicrobials is, therefore, limited. However, the use of antimicrobials as growth promoters should be reconsidered, because it is particularly this kind of use that appears to be closely associated with the most successful examples of transmission of resistance genes from farm animals to humans.

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MATHEMATICAL MODELLING OF PSEUDORABIES VIRUS (SYN. AUJESZKY'S DISEASE VIRUS) OUTBREAKS AIDS ERADICATION PROGRAMMES: A REVIEW

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SUMMARY

Pseudorabies virus will be eradicated from the Netherlands if a typical infectious pig (R_{ind}) infects, on average, less than one other pig. In this review, we used a stochastic SIR model to estimate R_{ind} using data from the field and from experiments. R_{ind} in sow herds was estimated to be significantly less than 1 and in rearing and finishing pigs R_{ind} was higher than 1. However, if R_{ind} is higher than 1, PRV can still be eradicated if one infectious herd infects less than one other herd during the period that the herd is infectious ($R_{herd} < 1$). Some future developments in Dutch pig husbandry (e.g. group-housing of sows) and possible risks after halting vaccination are also quantitatively evaluated.

INTRODUCTION

Pseudorabies virus (PRV, syn. Aujeszky's disease virus) is a herpesvirus which was first described by Aladar Aujeszky in 1902, when he distinguished pseudorabies from rabies (4). The severity of the clinical signs and the mortality rate as result of a PRV infection decrease with age. Young piglets unprotected by maternal antibodies develop nervous signs after infection and usually die. Older pigs (finishers) usually survive infection, although they may develop fever and respiratory signs; sows may abort (19,34). Swine are the natural hosts of the virus, but other species, such as cattle, dogs, and cats, can be infected as well and invariably die. In the Dutch situation, only pigs are sources of viral spread. In the USA raccoons are believed to be healthy carriers and in Europe antibodies have been found in wild boar (18,48).

The Dutch pig industry exports a vast number of live pigs, and this export may be hampered in the future by trade restrictions because of PRV infection. Therefore, in 1993, a campaign was started to eradicate PRV (43). When this campaign started, the virus was widespread (39). The campaign consisted of two phases. In the first phase, an intensive and systemic vaccination programme with efficacious g(lycoprotein)E²-negative vaccines was carried out. In the second phase, pigs were tested and gE-positive, thus infected, pigs were culled.

Infected pigs can be distinguished from vaccinated pigs if only gE-deleted vaccines are used. A so-called gE-ELISA can detect antibodies against this gE-antigen, which is absent in the vaccine strains (30,32). So far, all pathogenic PRV field isolates have been found to be gE-positive (33). Thus, infected animals, vaccinated as well as unvaccinated ones, test positive in this gE-ELISA, whereas all uninfected pigs, vaccinated as well as unvaccinated ones, test negative. In an eradication programme this distinction between infected and uninfected pigs is necessary, because PRV can become latent in infected pigs and after reactivation those pigs can become infectious again and can thus infect other pigs (1,9,36,46).

Generally, transmission of infectious agents in a population of individuals depends on the susceptibility of an individual, which is reflected by, among others, the dose needed to infect an individual, the infectivity of an individual after infection, which is reflected by, among others, the amount of virus excreted upon infection, and the rate and intensity of contacts between susceptible and infectious individuals. For eradication purposes, a vaccine has to reduce the susceptibility of a vaccinated pig, which means that the dose needed to infect a vaccinated pig is higher than needed to infect an unvaccinated pig

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² gE was formerly called gl.