EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

OPINION OF THE

SCIENTIFIC COMMITTEE ON VETERINARY MEASURES RELATING TO PUBLIC HEALTH

ON

THE HUMAN HEALTH RISK CAUSED BY THE USE OF FLUOROQUINOLONES IN ANIMALS

(adopted on 26-27 March 2003)

Table of contents

1. EXECUTIVE SUMMARY

Fluoroquinolones represent a complex group of synthetic broad-spectrum antimicrobials, widely applied in human and animal therapy. The risk to human health caused by the use of fluoroquinolones in veterinary medicine consists of the possible transfer of resistant zoonotic bacteria, particularly *Salmonella* spp. and *Campylobacter* spp., from animals to man, either by direct contact with animals or via the food chain. Epidemiological data have demonstrated an association between the (regional) introduction of fluoroquinolones into food animal therapy and the increasing prevalence of fluoroquinolone-resistant *Campylobacter* spp. in the respective human populations. The transfer of quinolone resistant zoonotic pathogens via the food chain is of emerging concern, as the use of fluoroquinolones in veterinary medicine has been associated with an increasing prevalence of resistant *Samonella* spp. and *Campylobacter* spp. in poultry meat and meat products. A high prevalence of fluoroquinolone-resistant foodborne pathogens affects the therapeutic possibilities and probably the efficacy of antimicrobial therapy in human medicine.

Salmonella spp. are common in poultry, pigs and cattle production, but can also be found in horses, companion (pet) animals, wild birds and rodents, as well as in reptiles. Animals infected with non-host adapted *Salmonella* spp. are usually a-symptomatic carriers that are continuously or intermittently shedding the bacteria into the environment. Due to their role as causative agents of foodborne diseases in humans, *Salmonella* spp. represent a challenge in modern animal husbandry. Reducing the prevalence of *Salmonella* in food commodities will also decrease the risk for the consumer to acquire resistant *Salmonella*.

Thermophilic *Campylobacter* spp. are widespread in nature as the principal reservoir is the alimentary tract of wild and domestic animals, and birds. In turn, thermophilic *Campylobacter* spp. are frequently isolated from poultry and poulty meat, cattle, pigs and sheep, as well as incidentally from companion animals. *Campylobacter* spp. easily acquire resistance to fluoroquinolones if these antimicrobials are used regularly in animals. As with *Salmonella* spp., *Campylobacter* spp. remain a challenge for modern animal husbandry, especially in poultry production.

As yet, no quantitative assessment of the risk to public health, related to fluoroquinolone-resistant *Salmonella* spp. and/or *Campylobacter* spp. infections can be presented.

However, to reduce the risk for foodborne infections and the possible transfer of fluoroquinolone resistant *Salmonella* spp. and *Campylobacter* spp. from animal to man, precautionary veterinary measures should be directed towards:

(I) the reduction of the prevalence of *Salmonella* spp. and *Campylobacter* spp. in food producing animals by implementing strict hygiene controls at the farm, during slaughter and transport, as well as at the retail and even consumer's level.

(II) the reduction of the overall use of fluoroquinolones by restricting their application to the selective use upon prescription of a licensed veterinarian.

(III) the implementation of a mandatory antimicrobial resistance monitoring in the EU.

Finally, the public at large should be informed adequately about the risk of transfer of fluoroquinolone-resistant *Salmonella* spp. and *Campylobacter* spp. from animals (including pet animals) to man and via the food chain.

2. BACKGROUND

Fluoroquinolones are an important group of antimicrobials used both in human and veterinary medicine and these substances are commonly used to treat salmonellosis and campylobacteriosis in humans. In the European Union fluoroquinolones are currently authorised for use in humans as well as in cattle, pigs, poultry and companion animals.

The Scientific Steering Committee issued an opinion on antimicrobial resistance (SSC, 1999). In this report the Committee concluded that the increasing prevalence of resistance to antimicrobial agents among pathogenic microorganisms, and particularly among bacteria, is now an important problem which has serious implications for the treatment and prevention of infectious diseases in both humans and animals. The Committee also stated that transmission to man of zoonotic agents, such as *Salmonella* spp. and *Campylobacter* spp., is of particular importance in assessing the relationship between resistance in animals and resistance among human pathogens.

Also the Committee on Veterinary Medicinal Products (CVMP) issued a report on the qualitative risk assessment of antimicrobial resistance in the European Union associated with therapeutic use of veterinary medicines on 14 July 1999. The Committee concluded among other things that in scientific literature evidence exists of transfer of resistant *Salmonella* and *Campylobacter* from animals to humans via the food chain and that the main public health risk are caused by these zoonotic bacteria.

Today there is an international concern about the increasing antimicrobial resistance in zoonotic agents, such as *Salmonella* and *Campylobacter*. This concern has been endorsed by the World Health Organisation (WHO), the Office International des Epizooties (OIE) and the Codex Alimentarius. The antimicrobial resistant zoonotic agents can form an additional health risk for humans due to possible failures in treatments of infections caused by them. Especially the emergence of multiresistant *Salmonella* strains and *Salmonella* and *Campylobacter* strains with reduced susceptibility to fluoroquinolones has become a cause for concern.

The Food and Drug Administration (FDA) in the USA has recently conducted a formal risk assessment on the human health impact of fluoroquinolone resistant *Campylobacter* in chickens. On the basis of this risk assessment the FDA is proposing to withdraw fluoroquinolones from the use in poultry. The Committee for Veterinary Medicinal Products (CVMP) has issued a reflection within the European context on this intention of the FDA to restrict the use of fluoroquinolone enrofloxacin in poultry. The CVMP agreed that fluoroquinolone resistant *Campylobacter* in poultry affects the therapeutic choice of medicines for human patients in risk, but found that *Campylobacter* infections are rarely treated with antimicrobials. However, the CVMP reminded that fluoroquinolones are also important drugs in veterinary medicine, and reducing the availability of medicines for veterinarians can result in animal health problems, which may also form a threat to human health.

3. TERMS OF REFERENCE

The Scientific Committee on Veterinary Measures relating to Public Health is requested to advise the Commission regarding the risk for human health caused by the use of fluoroquinolones in animals and specifically the consequent presence of fluoroquinolone resistant *Campylobacter* spp. and *Salmonella* spp. strains in the food chain.

In order for the Commission, to propose managerial options, the Committee is asked to consider the arguments "for" and "against" veterinary control measures from the point of view of public health.

4. FLUOROQUINOLONES

4.1. Mode of action

Fluoroquinolones comprise a group of synthetic antimicrobials, which have been derived from 1,4-dihydro-4-oxoquinoline-3-carboxylic acid. More than 10,000 compounds have been designed from the parent bicyclic 4-quinolone molecule. Initially, therapeutically applied fluoroquinolones had a carboxylic group in position 3, a keto group in position 4, fluorine in position 6, and a piperazinyl or methyl-substituted piperazinyl group (increasing anti-Gram negative and antipseudomonal activity) in position 7. Further modifications of the molecular structure involved substitutions at the N-1 position, enhancing activity against Gram-negative and Gram- positive bacteria and improving drug kinetics in mammalian species (for review see Neu, 1990; Grohe, 1998, Petersen and Schenke 1998). Recently, the group of 8-methoxy quinolones has been recognised, having an increased activity against Gram-positive bacteria and being effective also against wild-type and first-step *gyr A* mutants (Fung-Tomc *et al.*, 2001).

Cellular targets: Bacterial DNA gyrase (topoisomerase II), an enzyme involved in the supercoiling of the bacterial DNA and thus being essential for replication and transcription, has been identified as primary target for most fluoroquinolones (for review see Maxwell and Critchlow, 1998). Recently, DNA topoisomerase IV has been identified as second target. Topoisomerase IV is involved in the ATP-dependent relaxation of DNA and evidence suggests that Topoisomerase IV might be the primary target in certain bacteria such as *Staphylococcus aureus* and streptococci (Kaats and Seo, 1998).

Binding of fluoroquinolones to bacterial topoisomerase-DNA complexes will generally result in a bactericidal effect, which is concentration-dependent. A 100% bactericidal effect is achieved at drug concentrations exceeding 8 times the MIC (Minimum Inhibitory Concentration) (Maxwell and Critchlow, 1998). A paradoxical bacteriostatic effect might occur at extremely high concentrations (> 20 times MIC), presumably caused by inhibition or protein or RNA-synthesis.

The affinity of fluoroquinolones to bacterial gyrases is significantly higher than their affinity to eukaryotic DNA-topoisomerases, which explains their broad safety margin in human and animal therapy (Robinson *et al.*, 1991).

Antibacterial spectrum: The first generation of quinolones (nalidixic acid, flumequin, oxolinic acid) were effective particularly against Gram-negative bacteria (*Salmonella* spp. *E. coli, Bordetella* spp. and *Yersinia* spp.). With the introduction of enrofloxacin, the prototype of the second generation of quinolones (denoted fluoroquinolones), the spectrum was broadened towards Gram-positive bacteria *(*staphylococci, streptococci*, Listeria monocytogenes*) and includes also *Campylobacter* spp., *Pseudomonas aeruginosa* and *Mycoplasma* spp.*,* as well as anaerobic Gram-positive and Gram-negative bacteria. Recently introduced fluoroquinolones have an even improved efficacy, being designed to meet specific requirements of human and veterinary therapy (for review see Maxwell and Critclow, 1998).

4.2. Mechanisms of resistance to fluoroquinolones

As yet, available data, both molecular as well as clinical epidemiological data, indicate that acquired resistance to fluoroquinolones is not a trait that is easily transferable between bacteria. Most typically, resistance is based upon point mutations in the DNA gyrase gene (see below), and this kind of resistance mechanisms has so far not been shown to be transferable. No evidence for plasmid-mediated resistance has been found following the use of fluoroquinolones under clinical conditions. However, recently, transferable resistance to fluoroquinolones and nalidixic acid has been found in a clinical isolate of *Klebsiella pneumoniae* on a broad host range plasmid (Martínez-Martínez *et al.*, 1998). A distinct gene (*qnr*), which transfers quinolone resistance has been cloned and sequenced (Tran and Jacoby, 2002). From the alignment of the amino acid sequence of the gene product (Qnr) with related proteins, it seems that it could have evolved from an immunity protein designed to protect DNA gyrase, suggesting the possibility of a novel resistance mechanism to fluoroquinolones. However, the exact mechanism of resistance and its prevalence in clinical bacterial isolates remains elusive.

Commonly, resistance to fluoroquinolones occurs by three different mechanisms (reviewed by Everett and Piddock, 1998):

(1) mutations in the DNA gyrase gene:

In *Salmonella* spp, *E. coli* and many other Gram-negative bacteria, quinolone resistance is conferred by point mutations in the *gyrA* gene. All mutations described have been found to reside in the quinolone determining region (QRDR) of the A-subunit of DNA gyrase (topoisomerase II), i.e. amino acids 67-122. Amino acid changes at Ser-83 (to Phe, Tyr, or Ala) or at Asp 87 (to Gly, Asn, or Tyr) are the most frequently observed changes in nalidixic acid resistant strains. Double mutations in both residues Ser-83 and Asp-87 have been found in fluoroquinolone resistant clinical isolates of *E. coli* and *Salmonella* spp. (Heisig *et al.*, 1995; Everett, *et al.*, 1996; Griggs *et al.*, 1996; Deguchi *et al.*, 1997; Taylor and Chau, 1997). A QRDR has also been identified in the *gyrB* gene of *E. coli,* but the overall contribution of *gyrB*-mutations to fluoroquinolone resistance remains to be elucidated. In *Campylobacter* spp. species, being quinolone-resistant and isolated over a period of 5 years (1990-1995) from faeces of patients with enteritis in UK, mutations in the quinolone resistance-determining region of *gyrA* have been

found as well (codon 86). In contrast, mutations in *gyrB* were only observed in 8/192 isolates (Piddock *et al.*, 2003).

In Gram-positive bacteria (for example *Staphylococcus aureus*), topoisomerase IV, of which ParC and ParE are homologous to GyrA and GyrB, respectively, is the primary target for fluoroquinolones. Mutations in the genes *parC* and *parE* at positions equivalent to those identified in *gyrA* and *gyrB* participate in the high level resistance to fluoroquinolones (Reyba *et al.*, 1995; Vila *et al.*, 1996).

At present, topoisomerase IV has been recognised also as second target for quinolones in Gram-negative bacteria such as *E. coli*. Evidence suggests that *parC* mutations occur frequently when Gyr A is already resistant.

(b) Activation of efflux pumps: Efflux pumps are known to contribute to intrinsic and acquired resistance to many different antimicrobials. They reduce the intracellular accumulation of antimicrobials in bacterial cells, thus reducing antimicrobial activity. Genomic analysis has demonstrated the existence of multiple, putative efflux pumps in many bacteria as products of distinct genes (*emrAB, acrAB, norA, nfxB, nfxC* and others) (for review see Blackmore *et al.,* 2001). For example, high level fluoroquinolone resistance in *Campylobacter* spp. isolates from chickens were found to be conferred by activation of the CmeABC efflux pump (Luo *et al.*, 2003).

(c) Decrease in permeability: A decrease in the permeability of the bacterial cell wall caused by alterations in the hydrophilic pores (outer membrane porins) has been described as third mechanism of acquired resistance.

Point mutations (see (a)) result in high-level resistance which confers to the entire group of fluoroquinolones, with the exception of some recently developed substituted C8-methoxy fluoroquinolones.

Activation of the efflux pump as well as decreased cell permeability may also confer resistance to other antimicrobials such as tetracyclines and cephalosporins.

Finally, *E. coli* and a number of other microorganisms posses mechanisms which provide intrinsic protection against a wide range of chemically unrelated structures, including fluoroquinolones. This resistance has been linked to mutations of the *mar* locus. MAR A (Multiple Antibiotic Resistance protein A) is a transcriptional regulator protein activating the expression of different gene products, including those for known efflux pumps. MAR expression can be induced by certain antimicrobials such as tetracyclines and chloramphenicol, as well as by salicylates and other related compounds, and has found to emerge in different bacterial populations including *Enterobacteriaceae* (Cohen *et al.*, 1993). MAR expression (and over-expression) results in a phenotypic phenomenon denoted PAR (Phenotypic Antibiotic Resistance) conferring resistance to a number of antimicrobials including nalidixic acid, and reducing fluoroquinolone-killing capacity up to four times the MIC (Goldman *et al.*, 1996). This finding is not only of clinical relevance (in setting appropriate dosages for fluoroquinolones) but might also be of epidemiological relevance, as

resistance to nalidixic acid is a common marker in the monitoring of the prevalence of fluoroquinolone resistance.

4.3. Indications and dose regime

Fluoroquinolones are used to treat a variety of infections based on their broad antibacterial spectrum, both in human and animal medicine. Accepted indications for the therapeutic use of fluoroquinolones are infections of the urinary tract including prostatitis, infections of the gastrointestinal tract including traveller's diarrhoea, respiratory tract infections, intra-abdominal infections, and sexual transmittable diseases, as reviewed by Schacht (1998). Consequently, they can be used to treat patients infected with *Salmonella* spp. or *Campylobacter* spp. if the severity of the infections justifies antimicrobial therapy.

In the past, most fluoroquinolones have been licensed, both in human and veterinary medicine, in dosages derived from conservative studies, in which MIC values were compared with achievable plasma-concentrations following the intended route of administration. In this approach, as with other antimicrobials, the dose was established under the assumption that a plasma-concentration time profiles was achieved in which C_{max} (maximal concentration) exceeds twice the MIC_{90} value for the pathogen under consideration. Moreover, plasma-concentrations should be above MIC_{90} for half the intended dose interval.

However, already in 1987, Kaatz *et al.* reported that in an experimental model of *Staphylococcus aureus* endocarditis, when treated with ciprofloxacin, resistance occurred at concentrations 2 times the MIC value. Increasing the dose to 4 times the MIC decreased the incidence of resistant bacteria, and at 10 times the MIC, no resistance could be induced. Under field conditions, the MIC values for bacteria will vary and modern dose regimes aim to achieve tissue concentrations, which are sufficient to kill susceptible organisms taking into account the known variability of field strains. Application of the concept of concentration-depending bacterial killing should be integrated into pharmacodynamic (PD) and pharmacokinetic (PK) models (Stahlmann and Lode, 1998) estimating the optimal dose per animal species. This approach will improve clinical and bacterial outcome of therapy, while at the same time reducing the length of therapy; thereby also reducing the effect on the intestinal flora (Preston *et al.*, 1998a; Frimodt-Møller, 2002; Drusano *et al.*, 2002). Finally, implementing the concept of dose-dependent antibacterial activity will considerably reduce the emergence of resistant bacterial populations (Hyatt *et al.*, 1997, Pendland *et al.*, 2002). In contrast, application of fluoroquinolones at lower doses (sub-lethal to the pathogen) selects for resistant target bacteria. Since fluoroquinolone resistance is predominantly conferred by chromosomal mutations, resistant bacteria have the potential to persist, even after cessation of treatment, and to form a stable population, which might be transferred into the environment, to other animals or humans in contact with these animals and to animal derived foods.

Based on experimental data, originated from an integrated PK/PD approach, it is recommended (Hyatt *et al.*, 1995; Preston *et al.*, 1998b; Schentag and Scully, 1999) to apply doses in clinical therapy that result in

C_{max} : MIC >10 : 1

AUC_{0-24} : MIC > 125 : 1 = AUIC > 125

C_{max} = peak plasma concentration
MIC = minimum inhibitory concentration
AUC = area under the plasma-concentration versus time curve
AUIC = area under the plasma-concentration versus time curve to MIC ratio

Subsequently, therapy of uncomplicated infections can be achieved with a single dose (or in some cases by one repetition), which will reduce the absolute amount of antimicrobial used (Morissey, 1997; Fung-Tomc *et al.*, 2000). However, although it is generally recognized that fluoroquinolone action is concentration-dependent against Gram-negative bacteria, justifying the use of the above-mentioned surrogate markers AUC/MIC and Cmax/MIC, a time-dependent effect is assumed against some Gram-positive species. For time-dependent killing T > MIC (time above MIC) is considered to be a more relevant surrogate marker, and in clinical use both mechanisms have to be taken into account (Bosquet-Melou *et al.*, 2002, AliAbidi and Lees 2000, Lees and AliAbidi 2002).

5. CURRENT PRACTICE IN THE USE OF FLUOROQUINOLONES IN VETERINARY AND HUMAN MEDICINE

5.1. Veterinary medicine

In Europe, all veterinary medicinal products have to be licensed by the respective national or EU authorities (EMEA) prior to marketing. Details on the evaluation of the individual drugs especially relating to antimicrobials, including the assessment of potential resistance, are available on the EMEA Webpage (htpp://www.emea.eu.int) (EMEA, 2001a, b,c).

The introduction of (fluoro)quinolones as effective broad spectrum antimicrobials into veterinary medicine soon resulted in their widespread use. Prominent indications for their use, in veterinary medicine, are respiratory and enteric infections in farm animals. In cattle, this applies to *Pasteurella* spp*, Haemophilis somnus* and *Mycoplasma bovis*; in swine to *Pasteurella* spp*, Mycoplasma* spp*, Actinobacillus pleuropneumonia* as well as *E. coli* . . Poultry (broilers) and turkeys are treated with fluoroquinolones in cases of infections caused by *Pasteurella* spp*., Mycoplasma* spp. and *E. coli* but also with the aim to reduce the prevalence of *Salmonella* spp. and *Campylobacter* spp. in the flock. The latter applications are not justified, as clinical outbreaks of Salmonellosis and/or Campylobacteriosis are rare. In fish, septicaemia and skin ulcers (*Aeromonas hydrophila*, *Aeromonas salmonicida* and *Vibrio* spp) are the major indications. In companion animals, fluoroquinolones are considered optimal in the treatment of prostatitis (in dogs), recurrent pyodermatitis (*S. intermedius*), and other

infections (respiratory tract and urinary tract), which require the use of a drug with a large volume of distribution. Moreover, fluoroquinolones are regularly used in exotic animals (Prescott *et al.*, 2000).

Available data suggest that in certain countries fluoroquinolones are not only applied as therapeutic agents, but are also used for disease prevention. Quinolone production and usage is estimated to be about 50 tonnes for proprietary products (mainly USA, EU, Japan, South Korea). In addition, because of their low prices, about 70 tonnes of generic quinolones seem to be produced. Thus, available data on actual usage are known to be grossly incomplete, particularly for non-proprietary quinolones. For instance, data from China estimate annual quinolone consumption in animals, in China alone, to be in the range of 470 tonnes (WHO, 1998). Data from Thailand (WHO, 2002), show that fluoroquinolones are the most popular antimicrobials for disease prevention (prophylaxis) and treatment, both in broiler farms and breeding farms. This widespread use is likely to promote development of fluoroquinolone resistance in *Salmonella* spp. and *Campylobacter* spp. This again poses a risk to Europeans following consumption of chicken products when visiting Thailand as well as through the import of poultry products from Thailand (WHO, 2002). There is reason to believe that this situation applies also to other countries.

In USA, two fluoroquinolones were approved for use in poultry in 1995 and 1996 to control poultry mortality resulting from *E. coli* infections. Near the time of approval, the FDA instituted several strategies intended to prevent, or mitigate, the development of resistance. Among others, FDA demanded that these drugs should only be used by veterinarians or on prescription of a licensed veterinarian. Moreover, FDA issued regulations, which became effective in August 1997, prohibiting all off-label use of fluoroquinolones in animals. However, fluoroquinolone resistance still rapidly increased among *Campylobacter* spp.. Therefore, FDA concluded that the agency's attempts to prevent the development of fluoroquinolone resistant human pathogens through limiting these drugs in veterinary medicine to prescription-only use, might not be sufficient to avoid an increasing prevalence of resistant *Campylobacter* spp. strains (FDA, 2000).

Within the European Community, enrofloxacin is licensed for all food producing species, whereas marbofloxin is licensed only for bovine and porcine species and for horses, danofloxacin for bovines, porcines and for chickens, whereas difloxacin in mainly used in poultry (chicken, turkeys), and sarafloxacin in chickens and in fish. These and other fluoroquinolones are also used in small animal practice (for details see EMEA report, 1999 Annex II, and individual product reports http:/www.emea.eu.int).

As in the USA, the introduction of fluoroquinolones into veterinary therapy has been accompanied by an increase in resistant human pathogens (for details see chapter 6). Whereas some EU member states (for example the Nordic countries and the Netherlands) have implemented an active policy to limit the use of fluoroquinolones used in veterinary medicine (see also chapter 8), other European (and non-European) countries have not taken any precautions yet. In those countries having implemented guidelines for the prudent use of antimicrobials, the prevalence of resistant microorganisms

seems to stabilize or even decrease in the animal populations (including poultry). Moreover, implementation of the new CVMP guidelines, taking into account the recent knowledge on pharmacodynamics of antimicrobials (dose-dependent *vs* time-dependent effects) are believed to further reduce fluoroquinolone resistance in livestock (http:/www.emea.eu.int).

5.2. Human medicine

As in veterinary medicine, fluoroquinolones are widely used in human medicine (see EMEA, 1999, Annex 2 Table 11 for licensed products). As mentioned before, they are considered as very effective and safe drugs in the treatment of urinary tract infections, including prostatitis, infections of the gastrointestinal tract including traveller's diarrhoea, respiratory tract infections, intra-abdominal infections, shigellosis and the treatment of sexual transmittable diseases (Schacht, 1998). Campylobacteriosis and Salmonellosis, could be treated successfully with fluoroqinolones as well, but it is generally accepted that antimicrobial treatment is usually not required for enteritis of moderate severity.

Major indications for which no obvious alternatives exist in human medicine are the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections (ciprofloxacin, often in combination with rifampicin), despite the fact the fluoroquinolone resistance has been found increasingly in MRSA, and enteric fevers caused *by Salmonella* Typhi and *Salmonella* Paratyphi as well as infections caused by multi-drug resistant *Mycobacterium tuberculosis* (Kuhlmann *et al.*, 1998).

However, simple observation of the current medical practices revealed that fluoroquinolones are even used for minor (recurrent) infections, in cases where no proper diagnosis has been made, and as convenience antimicrobials for patients at risk (for example during travelling), obviously not matching the criteria set for the prudent use of antimicrobials.

Limited data are available regarding the occurrence of fluoroquinolone resistance in human pathogens (Critchley *et al.*, 2002; Hopper, 2002; Jones, 2002; Zhanel *et al.*, 2003) other than the zoonotic bacteria *Salmonella* spp. and *Campylobacter* spp. (for the latter organisms see chapter 6.1.2 and 6.2.2.). In a hospital setting, it is generally assumed that even in cases where fluoroquinolone resistant *Campylobacter* spp. and *Salmonella* spp. in individual patients have been detected, resistant organisms are not likely to be transmitted between patients due to hygienic barriers. This is in contrast to MRSAs that are more easily spread between patients (FDA, 2000).

5.3. Conclusions

Fluoroquinolones are regularly prescribed drugs in human and veterinary medicine. Their favourable kinetics (large volume of distribution) and broad antibacterial activity account for their therapeutic value in the treatment of infections, where common antimicrobials fail to reach appreciable effective concentrations in target tissues. Moreover, their efficacy in cases of multi-resistant pathogens (including MRSA) underlines their therapeutic value in clinical practice. At the same time, however, the intensive use in clinical therapy increases the risk for development of resistance to fluoroquinolones,

particularly following inappropriate dosing schedules. Recent strategies aim on reducing the risk of fluoroquinolone resistance by limiting their use and improving the dose regime thereby reducing the duration of administration.

6. *SALMONELLA* SPP. AND *CAMPYLOBACTER* SPP.

6.1. *Salmonella* spp.

6.1.1. Epidemiological aspects

The natural reservoir of *Salmonella* spp. is the intestine of animals, including humans. *Salmonella* spp. can, however, be isolated from very different sources. The bacteria are robust, and can survive in the environment, including the food-processing environment, and in feed and foods for prolonged periods (ICMSF, 1996). If conditions are favourable, Salmonellae are able to grow outside the host. More information about survival, growth and inhibition is given in the Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on Salmonellae in Foodstuffs (SCVPH, 2003a)

Salmonella spp. can be found among a wide range of animals, both wild and domestic, ranging from mammals and birds to reptiles and insects. Some *Salmonella* serovars are host-specific, e.g. *S.* Typhi in humans, *S.* Pullorum in poultry and *S.* Abortus-ovis in sheep, but most can be isolated from a large number of animals, including man. Animals infected with non-host adapted *Salmonella* spp. are usually asymptomatic carriers that continuously or intermittently shed the bacteria into the environment.

Salmonella spp. are common in pig, poultry and cattle production as well as in horses, and, typically, the animals are healthy carriers. The bacteria are spread among farm animals through contact with other farm animals, wild birds or rodents, through contaminated feed or water, or through infected utensils, equipment or workers. Due to their role as a cause of foodborne disease in humans, *Salmonella* spp. represents a challenge in modern food animal production. Prevalence levels of *Salmonella* spp. in food animals vary depending on climatic factors as well as on how stringent control measures are introduced. Control measures include, amongst others, eradication of all positive holdings or breeder populations, isolation of positive holdings, vaccination programme, and feed control.

The transmission of *Salmonella* spp. to humans may be quite complex. Although person-to-person spread of *Salmonella* spp. may be common in developing countries, this route is rare in developed countries, where most infections are foodborne. The bacteria may contaminate all stages of food production, however, primary production of food animals remains the most important reservoir of *Salmonella* spp. entering the human food chain. Due to their widespread occurrence among food animals such as poultry, pigs, cattle, and game birds, *Salmonella* spp. may frequently be encountered in raw food products from such sources. Nevertheless, *Salmonella* spp. are also an important contaminant of other food commodities such as drinking water and fresh produce that can be contaminated by faecal material of human or animal origin. The Opinion of the Scientific Committee on Veterinary

Measures relating to Public Health on Salmonellae in Foodstuffs reviews prevalence data from primary production and various food categories in Europe (SCVPH, 2003a).

Salmonella spp. are commonly isolated from reptiles including turtles, As such animals may be kept as pets, they represent a risk for spread of *Salmonella* spp. to humans. In Sweden, transmission from reptiles to children represents a significant source of domestic *Salmonella* spp. infections in human (http://www.sva.se/pdf/zoonosinsweden.pdf).

Salmonellosis occurs both as sporadic disease and as outbreaks. Previously, it was believed that 10^5 to 10^7 bacteria are required to establish a non-typhoid salmonellosis (McCullough and Eisele, 1951). However, data from recent outbreaks of foodborne diseases indicate that infections can be caused following ingestion of as few as 10-45 cells, especially among immuno-compromised persons or the elderly or the very young (D'Aoust *et al.*, 1985; Lehmacher *et al.*, 1995).

Any serovar that is not host-adapted is considered capable of causing gastro-intestinal illness of varying severity in humans. The most frequent reported serovars involved in salmonellosis in the EU are *S.* Typhimurium and, especially in more recent years, *S*. Enteritidis, particularly Phage Type 4 (PT4), (ACMSF, 2001; FAO/WHO, 2001; EC, 2002). *S.* Enteritidis and *S.* Typhimurium were also the most frequently reported serovars involved in outbreaks of salmonellosis in Europe in the period 1993-1998, occurring in a ratio of approximately 3:1 (FAO/WHO, 2001). In 2000, the EC reported that the most frequently reported serovars in humans, based upon reports from nine countries, were *S.* Enteritidis (59.1%), *S*. Typhimurium (13.0), *S.* Hadar (1.8%), *S*. Virchow (1.4%), *S.* Infantis (0.9%), *S.* Agona (0.8%), *S.* Brandenburg (0.7%), and *S.* Newport (0.5%) (EC, 2002). In 2000, *S.* Typhimurium DT 104 was the most commonly reported phagetype of *S.* Typhimurium (EC, 2002).

6.1.2. Occurrence of fluoroquinolone resistance

Antimicrobial resistant *Salmonella* spp. in animal production have been reported since the 1960s (Swann, 1969). In general, the occurrence of resistance seems to have increased over the years, and is associated with the selective pressure exerted by the use of antimicrobials (Cohen, 1992). However, there are large variations between regions, sectors, and sources. Moreover, the tendency to acquire resistance seems to vary between different serovars (EC, 2003).

In many studies, the occurrence of resistant strains in animals or food of animal origin have been directly linked to the use of antimicrobials in primary production (Spika *et al.*, 1987, Holmberg *et al.*, 1984a, Dunne *et al.*, 2000, Fey *et al.*, 2000). An investigation of 52 *Salmonella* outbreaks in the USA between 1971 and 1983, revealed that food-producing animals were the source of 18 (47%) of the 38 outbreaks with identified sources, and that these cases also represented 11 (69%) of 16 resistant outbreaks strains and 6 (46%) of 13 susceptible outbreak strains (Holmberg *et al.*, 1984b).

In general, antimicrobial resistant *Salmonella* sp. are commonly isolated from various sources throughout Europe (EC, 2003). Over the last decade, strains of *S. enterica* with multiple drug resistance have been distributed widely in many European countries, in particular multi-resistant clones of *S.* Typhimurium DT104 and 204b. In 2000, 40% of 27059 human clinical isolates of *Salmonella* tested were resistant to at least one antimicrobial, with 18% exhibiting multiple resistance (to four or more antimicrobial agents) (Threlfall *et al.*, 2003).

Several studies have shown that resistance to nalidixic acid and decreased susceptibility to fluoroquinolones has increased among a variety of zoonotic *Salmonella* spp. from food animals and infections in humans (Heurtin-Le Corre *et al.*, 1999; Prats *et al.*, 2000; Threlfall *et al.*, 1997, 1999a,b). This has been linked to the authorization of fluoroquinolones for food producing animals in the UK, USA, and Denmark (WHO, 1998). There is uncertainty about the relative contribution of direct selective pressure versus the spread of resistant strains in the presence or absence of quinolone with respect to the emergence and dissemination of quinolone resistant *Salmonella* spp. (WHO, 1998).

Resistance to different types of antimicrobials, including fluoroquinolones, has become quite common among *S.* Typhimurium and many strains are multi-resistant (EC, 2003). In 2001, nalidixic acid resistant *S.* Typhimurium were reported from cattle (Belgium 5%, France 10%, and UK 10%), from pigs (Belgium 1%, Denmark 1%, France 2%, UK 10%, Italy 17%, Portugal 7 of 10 isolates), and poultry (Netherlands 3%, Austria 7%, Belgium 8%, France 19%, UK 28%). France also reported enrofloxacin resistant isolates from cattle (2%) and poultry (2%), and Austria ciprofloxacin resistant isolates from poultry (4%). Belgium, Denmark, The Netherlands, and Norway reported nalidixic acid resistance in 1-4% of human isolates (EC, 2003).

In several European countries as well as North-America, a multi-resistant clone of *S.* Typhimurium phage type DT104 (MR-DT104) became epidemic during the 1990s. MR-DT104 has been isolated from many different food animals including cattle, pigs, sheep, and poultry. Infection in humans is generally foodborne. MR-DT104 typically expresses resistance to ampicillin, streptomycin, chloramphenicol, tetracyclines, and sulfonamides. In the late 1990s, resistance to quinolones has been increasing in MR-DT104 isolates. In UK, the emergence of quinolone-resistant MR-DT104 in chicken, cattle, pigs, and humans followed soon after the licensing of enrofloxacin for animal use (EMEA, 1999). In 2001 in UK, overall 19,8% of DT104 and 104B isolates were resistant to nalidixic acid, an increase compared to 11,7% in 2000 (EC, 2003). Subsequent to the introduction of fluoroquinolones for food animal use in Germany in 1988, the emergence of fluoroquinolones resistant variants of multiresistant *S.* Typhimurium DT204c was observed. Resistance reached a prevalence of 50% in isolates from calves in a defined area of the country. In the years thereafter, the prevalence of resistant strains decreased, but data associating this change in prevalence with changes in fluoroquinolone usage in animals are not available (WHO, 1998). In contrast to *S.* Typhimurium, *S.* Enteritidis isolates are, in general, susceptible to most antimicrobials (EC, 2003).

However, resistance to quinolones is emerging in many countries (Mølbak *et al*., 2002; EC, 2002). In 2001, detection of nalidixic resistant isolates from poultry were reported from Austria (17%), France (9%), Greece (23%), the Netherlands (6%), and Portugal (60%) (EC 2003). In Portugal, enrofloxacin resistance was detected in 28% of the isolates tested, an increase from 10% as compared to 2000 (EC, 2003). Denmark and UK reported also findings of nalidixic acid resistant *S.* Enteritidis from poultry meat (EC, 2003).

In 2001, several European countries reported high percentages of nalidixic acid resistance in *S.* Hadar, *S.* Heidelberg, *S.* Infantis, *S*. Montevideo, *S.* Saintpaul, *S.* Virchow, and *S.* Kottbus, most isolates being derived from poultry. Belgium reported that 92% of human isolates of *S.* Hadar were nalidixic acid resistant. France reported findings of enrofloxacin resistant *S*. Hadar, *S.* Heidelberg, and *S.* Kottbus from poultry (EC, 2003).

Since 1996, the incidence of multiresistant *S.* Newport infections in humans has increased significantly in the US, and a specific ceftriaxone resistant type, *S.* Newport MDR-AmpC, is considered to be responsible for a significant percentage of the human cases. Data suggest that cattle, particularly dairy cattle, might be a source for human *S.* Newport MDR-AmpC infection in the US (MMWR, 2002).

In the Netherlands, *Salmonella* Paratyphi B var. Java increased in poultry from less than 2% of all isolates before 1996 to 60% in 2002. Despite the high exposure to contaminated poultry meat is high, human patients with Java infection are rare (0.3% of all isolates) However, 50% of the human isolates showed PFGE profiles identical to the poultry clone. Resistance to flumequin in *S.* Paratyphi B var. Java from poultry increased from 3% between 1996-2000 to 19% in 2001, and 39% in 2002, while that of other serovars in poultry remained at about 7%. *S.* Paratyphi B var. Java is becoming less sensitive to ciprofloxacin (van Pelt *et al.*, 2003).

6.2. *Campylobacter* spp.

6.2.1. Epidemiological aspects

Thermophilic *Campylobacter* spp. are widespread in nature (Jones, 2001). The principal reservoirs are the alimentary tracts of wild and domesticated mammals, and birds. This implies that thermophilic *Campylobacter* spp., especially *C. jejuni* and *C. coli* are commonly isolated from water sources, food animals such as poultry, cattle, pigs, and sheep, as well as from cats and dogs (Jones, 2001; FAO/WHO, 2002). In animals, *Campylobacter* seldom cause disease (WHO, 2000). Table 1 and 2 show some data on the prevalence of thermophilic *Campylobacter* spp. in food animals in Europe. It should be noted that the prevalence does not reflect disease. Foodstuffs, including poultry, beef, pork, other meat products, raw milk and milk products, and, less frequently, fish and fish products, mussels and fresh vegetables can also be contaminated (Jacobs-Reitsma, 2000).

Human campylobacteriosis is considered to be a zoonosis (WHO, 2000). *Campylobacter* spp. may be transferred to humans by direct contact with contaminated animals or animal carcasses, or indirectly through the ingestion of contaminated food or drinking water (FAO/WHO, 2002). While

transmission routes in low-income societies are complex and multifactorial, campylobacteriosis in the industrialised countries is primarily a foodborne disease, with poultry as a principal source (Friedman *et al.*, 2000; WHO, 2001). Table 2 summarises some data regarding the prevalence of thermophilic *Campylobacter* spp. in poultry meat in EU member states, which have implemented a monitoring system. Other sources of infections include undercooked meats and meat products, raw milk and milk products, and contaminated water or ice.

Common-source outbreaks account for a rather small proportion of cases, and the vast majority of reported cases are sporadic (WHO, 2000). Direct spread from one person to another can occur, but is uncommon in industrialised countries. These cases are believed to be sporadic, accounting for only a rather small proportion of the total number of reported cases (Friedman *et al.*, 2000).

Many cases of campylobacteriosis are associated with foreign travel (EC, 2002). Between 10-50% or more of all reported cases, depending on the country, result from the consumption of contaminated food or water in the countries visited.

Typically, more than 90% of the isolates from human campylobacteriosis cases are identified as *C. jejuni* (EC, 2002). In Northern Europe, a seasonality in the incidence of campylobacteriosis can be observed with a peak during the summer season (EC, 2002).

Table 1. *Campylobacter* spp. in broilers and products thereof in countries, conducting a monitoring programme (EC, 2002)

	1998		1999		2000	
Country	"n"	% pos.	"n"	% pos.	"n"	% pos.
Broilers (flock based data)						
Denmark	5943	47.1	6557	46.0	6160	37.9
Finland	NR	NR	1132	4.0	1094	5.8
Sweden	3561	9.1	3846	9.2	3969	9.9
Netherlands	189	30.7	151	16.6	128	24.2
Northern Ireland	NR	NR	194	21.6	NR	NR
Poultry meat (at processing plants)						
Belgium	-	-	-	-	171	28.7
Ireland	NR	NR	NR	NR	3422	53.9
Poultry meat (at retail)						
Austria	NR	NR	NR	NR	200	20.0
Belgium	-	-	139	57.6	83	7.2
Denmark	819	28.8	994	34.0	708	41.1
Finland	114	11.4	147	4.1	161	10.6
Germany	NR	NR	NR	NR	958	19.5
Ireland	NR	NR	NR	NR	391	38.9
Sweden	83	4.5	94	24.5	858	9.3
Netherlands	1009	26.9	859	23.5	1454	30.5
Norway [1]	NR	NR	101	8.9	-	-
Norway [2]	NR	NR	133	12.8	62	12.9

"n" numbers investigated. [1]Domestic broiler meat. [2]Imported broiler meat.

NR= no reported data

Table 2. *Campylobacter* spp. in cattle and pigs in countries, conducting a monitoring programme (EC, 2002)

	1998		1999		2000	
Country	"n"	% positive	"n"	% positive	"n"	% positive
Cattle (herd based data)						
Denmark	85	47.1	84	50.0	90	61.1
Netherlands	192	48.4	225	20.0	703 [1]	1.6
Norway	-	-	128	35.9	73 [1]	11.0
Pigs (herd based data)						
Denmark	318	68.6	312	53.5	310	64.2
Netherlands	38	97.4	190	45.3	-	-
United Kingdom	-	-	-	-	860	94.5
Sheep and lamb (flock based data)						
Denmark	-	-	137	24.8	-	-
United Kingdom	NR	NR	NR	NR	973	17.0
Netherlands	NR	NR	NR	NR	104	13.0

"n" numbers investigated. [1] Submissions for diagnostic purposes. [2] Survey. NR = no reported data.

6.2.2. Occurrence of fluoroquinolone resistance

Fluoroquinolone resistant *C. jejuni* were recognised during the late 1980s in Europe (Nachamkin *et al.*, 2002). Studies from several countries have shown the relationship between the approval of fluoroquinolones for use in food producing animals, and the development of fluoroquinolone resistance in *Campylobacter* spp. in animals and humans. As poultry is considered the principal source of *Campylobacter* spp. infections in industrialised countries, and human-to-human transmission is uncommon, it may be assumed that the contribution from the poultry reservoir plays the leading role in the emergence of fluoroquinolone resistance in *Campylobacter* spp. (Smith *et al.*, 1999).

The approval and use of fluoroquinolones in poultry in the Netherlands (Endtz *et al.*, 1991; Piddock, 1995) and Spain (Velázquez *et al.,* 1995) was followed by increases in fluoroquinolone resistance in *Campylobacter* spp. in treated animals and human patients. In the Netherlands, no fluoroquinolone resistant campylobacters were observed in 1982. In contrast, in 1989, two years after the licensing of enrofloxacin for veterinary use in 1987, 11% of human isolates and 14% of poultry isolates were fluoroquinolone resistant (Endtz *et al.*, 1991). In Spain, fluoroquinolone resistant *Campylobacter* spp. were practically non-existent until 1988 (Sàenz

et al., 2000). In a 1997-1998 study, an extremely high prevalence of ciprofloxacin resistance was detected among *Campylobacter* spp. strains, particularly those isolated from broilers and pigs (99%), with a slightly lower result for human isolates (72%). Cross-resistance with nalidixic acid was almost always observed (Sàenz *et al.*, 2000).

Also in the UK, increases in fluoroquinolone resistant pathogens in humans have been associated with approval of fluoroquinolones for use in poultry (Threlfall *et al.*, 1999a). A UK study of raw meat and poultry at retail sale, conducted in 1998, revealed the following *Campylobacter* spp. contamination rates: chicken meat 83%, lamb liver 73%, pig liver 72%, and ox liver 54%. Within the *C. jejuni* isolates originating from chickens, 10.8% were found to be fluoroquinolone-resistant, whereas up to 5.6% of the *Campylobacter* spp. isolates from other sources were fluoroquinolone-resistant. Among human isolates, 6% of *C. jejuni* and 25% of *C. coli* were fluoroquinolone-resistant (Kramer *et al.*, 2000). In Northern Ireland, fluoroquinolone-resistance in human isolates rose from <4% in 1992 to 17% in 2000, but there was no parallel rise in resistant isolates from locally produced poultry (Moore, 2001). The higher incidence of resistance in human isolates was attributed to the trend to consume poultry meat in preference to beef, and the increased importation of poultry products.

In Ireland, in the period 1996-1998, 3.1% of the poultry *Campylobacter* spp. isolates were ciprofloxacin resistant, whereas resistance was not found in human isolated. In 2000, 34% of *Campylobacter* spp. isolates from humans were ciprofloxacin resistant. Among samples of domestically produced (N=37) and imported (N=8) poultry, 19% and 75%, respectively, gave rise to ciprofloxacin resistant *Campylobacter* spp. isolates. None of the humans from whom ciprofloxacin resistant *Campylobacter* spp. were isolated, had been treated recently with ciprofloxacin (Lucey *et al.*, 2002).

In the US, two fluoroquinolones were approved for poultry use in 1995 (sarafloxacin) and 1996 (enrofloxacin) (FDA, 2000). Prior to 1995, there was very little, if any, fluoroquinolone-resistant *Campylobacter* in the US among domestically acquired foodborne disease. Since then, fluoroquinolone resistant *Campylobacter* spp. have been isolated from human clinical cases, poultry and poultry meat (FDA, 2000). In 1998, 9.4% of *C. jejuni* isolated from chicken carcasses, were fluoroquinolone resistant. In a US study in 1999, *Campylobacter* spp.resistant to nalidixic acid and fluoroquinolone were isolated from 32% and 24% of retail chickens, respectively (FDA, 2000). In a Pennsylvanian study involving human clinical cases, fluoroquinolone resistant *C. jejuni* was not observed in the period 1982-1992. However, resistance increased remarkably to 40.5% in 2001 (Nachamkin *et al.*, 2002). In Minnesota, the percentage of fluoroquinolone resistant *C. jejuni*, isolated from humans, increased from 1.3% in 1992 to 10.2% in 1998. The percentage of resistant infections that were acquired domestically increased from 0.3% in 1996 to 3% in 1998, and this increase was attributed to the use of fluoroquinolones in poultry (Smith *et al.*, 1999). In 2000, 14.2% of *C. jejuni* submitted to the Centre for Disease Control and Prevention in the National Antimicrobial Resistance Monitoring System (NARMS) were fluoroquinolone resistant (Nachamkin *et al.*, 2002). Preliminary data for 2001 show an increase up to 19.2% (K. Mølbak,

personal communication). The estimated number of individuals in USA that acquire fluoroquinolone resistant *Campylobacter* spp. infections, associated with the consumption of chicken products and subsequently receiving fluoroquinolone therapy, was 9,261 (95% confidence interval 5227-15326) in 1999 (FDA, 2001).

A Norwegian study in 2001 assessed the prevalence of fluoroquinolone resistance among a representative and random sample of *C. jejuni* isolates from 129 imported and 84 endogenous, sporadic human cases of campylobacteriosis, and from 113 broilers, each representing one broiler farm (Kruse and Skov Simonsen, 2002). Among the imported isolates, 60% were resistant to ciprofloxacin as opposed to 7% for the indigenous human isolates. The resistance prevalence for indigenous human isolates were comparable with data from the poultry isolates, where 96% were susceptible to all antimicrobials included, and only 2.7% resistant to enrofloxacin (and nalidixic acid) (Kruse *et al*., 2002). The use of antimicrobials in food producing animals in Norway has always been restricted, and very small amounts of fluoroquinolones are currently being used.

In Denmark, fluoroquinolone resistance in *Campylobacter* spp. isolated from humans increased from 10% in 1996 to 28% in 2000. In 2001, the prevalence dropped to 22% (DANMAP, 2002). The prevalence was highest in travellers returning from the Mediterranean countries, and from South and South East Asia, while the prevalence in indigenous cases was 16%. In Denmark, the use of fluoroquinolones in the food production is very limited, but about 1/4 to 1/3 of broiler chickens and other poultry products are imported. It remains to be determined whether these imported carcasses and products are a major source of indigenously acquired fluoroquinolone-resistant *Campylobacter* spp. infections (K Mølbak, pers. comm.).

7. PUBLIC HEALTH IMPLICATIONS RELATING TO FLUOROQUINOLONE RESISTANT *SALMONELLA* SPP. AND *CAMPYLOBACTER* SPP.

7.1. General aspects

The public health risks arising from resistance in zoonotic *Salmonella* spp. and *Campylobacter* spp. are increased morbidity, increased mortality, and increased costs to society associated with disease. The increased morbidity and mortality is often a result of treatment failures and may be preceded by prolonged duration of infections, increased hospitalization rates, increased risk for invasive infections and bacteraemia, and increased risk for complications and sequelae.

In industrialized countries, the occurrence of foodborne infections in humans caused by fluoroquinolone resistant *Salmonella* spp. and *Campylobacter* spp. is associated very often with the usage of fluoroquinolone in food animals. However, it is noteworthy that fluoroquinolone resistance may also develop in the patient during a course of fluoroquinolone treatment. In developing countries, where direct of indirect person-to-person transmission is more common, the human usage of fluoroquinolone plays a more prominent role with regard to fluoroquinolone resistant *Salmonella* spp. and *Campylobacter* spp.

The incidence of resistant *Campylobacter* spp. and *Salmonella* spp. infections are influenced by imported food commodities, and by infections acquired abroad during travelling, as clearly indicated in a Norwegian study (Kruse *et al.,* 2002). This globalisation of human mobility and food trade represent a challenge in regard to risk management in individual countries and in the EU to combat antimicrobial resistance.

7.2. *Salmonella* spp.

Salmonellosis in humans can induce several clinical syndromes, including enteric (typhoid) fever, localised entero-colitis and systemic infections by non-typhoid microorganisms. Infections with non-typhoid *Salmonella* spp. commonly result in entero-colitis that appears 8 to 72 h after ingestion. This condition is generally self-limiting, and remission of the characteristic non-bloody diarrhoeal stools and abdominal pain usually occurs within 5 days after onset of symptoms. Infections with non-typhoid strains can also progress to systemic infections and precipitate various chronic conditions such as reactive arthritis, Reiter's syndrome and ankylosing spondylitis (D´Aoust, 1991, 1997, 2000). Enteric fever and septicaemia due to *Salmonella* spp. are serious human illnesses, but occur seldom in the EU (Mølbak *et al.*, 2002).

Throughout the European Union, the reported incidence of salmonellosis in humans is quite high; up to 138 cases per 100,000 inhabitants (Belgium) (EC 2002). However, it should be noted that the national figures are based upon different monitoring systems and thus cannot be directly compared. Furthermore, the reported incidences present only the tip-of-the-iceberg due to underreporting, and it is estimated that the true incidence is 3.2-38 times higher (Mead *et al.*, 1999; Wheeler *et al.*, 1999). The common serovars of *Salmonella* spp. cause bacteraemia in 0.5-2.5% of culture-confirmed salmonellosis cases in the UK, and in less than 6% in the USA. Untreated or ineffectively treated *Salmonella* spp. bacteraemia in humans can be fatal (WHO, 1998).

Fluoroquinolones are important drugs for the treatment of extra-intestinal infections caused by *Salmonella* spp. (WHO, 1998), including enteric fever caused by *S.* Typhi and *S.* Paratyphi (ACMSF, 1998). Treatment of serious susceptible enteric infections with an effective fluoroquinolone can reduce the duration of illness and most likely prevent complications and adverse outcomes, including hospitalisation (FDA, 2000). Thus, the increasing emergence of quinolone resistance in *Salmonella* spp. is of public health concern.

A major risk factor for infections with antimicrobial resistant *Salmonella* spp. is previous treatment with antimicrobials (for any reason) and this association may result in increased incidence and illness severity (Helms *et al.*, 2002, Holmberg *et al.*, 1984b, Spika *et al.*, 1987, WHO, 1998). In a meta analysis, Barza and Travers (2002) showed that persons treated with antimicrobials prior to exposure had a 3.7 higher (95% Confidence Interval, 2.7-5.0) risk for infection with resistant *Salmonella* spp. as compared to non-treated individuals. Glynn *et al.*, (2003) found that antimicrobial treatment

for four weeks prior to infection was associated with a 5.7 times increased risk for MR DT104 infection (95% Confidence Interval –1.8-17.4).

Although there is no microbiologically proven link between antimicrobial resistance and the virulence of zoonotic *Salmonella* spp., increased rates of hospitalisation have been reported for patients having acquired infections with *S.* Typhimurium MR-DT104. Helms *et al.* (2002) found that patients infected with *S.* Typhimurium resistant to nalidixic acid had a 10.3 times (95% CI 2.8-37.8) higher mortality rate than the average Danish population. In contrast, patients infected with fully susceptible *S.* Typhimurium and penta-resistant *S.* Typhimurium who were 2.3 and 4.8 times, respectively, more likely to die. Pathogen resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracyclines, and nalidixic acid was associated with an excess mortality rate of 13.1 (95% CI 3.3-51.9). Quinolone resistant penta-resistant *S.* Typhimurium represented excess mortality as compared to quinolone-susceptible penta-resistant *S.* Typhimurium. Two studies based on outbreaks of resistant *Salmonella* spp. in the US and the UK have found case fatality rates of 4.2% and 3%, respectively (Holmberg *et al.*, 1984a,b; Wall *et al.*, 1994). In several studies, antimicrobial therapy for reasons other than enteric disease has been identified as a general risk factor for the acquisition of foodborne diseases for several reasons. Patients receiving antimicrobial treatment may already be vulnerable due to an underlying disease and, consequently, may be more susceptible to infection. This may be the underlying reason for an apparent difference in virulence between antimicrobial-resistant and antimicrobial-sensitive strains. Furthermore, antimicrobial treatment can alter the composition of the normal intestinal flora, which may result in a lower infectious dose of a pathogen needed to establish an infection. It is also possible that antimicrobial therapy in a patient harbouring resistant opportunistic bacteria, or with a sub-clinical infection caused by a resistant foodborne pathogen, may trigger development of clinical disease (CCFH, 2000).

7.3. *Campylobacter* spp.

Campylobacter spp. has been recognised as a cause of diarrhoea in man since 1972. In most industrialised countries, the reported incidence of campylobacteriosis has increased during the last decade, and for many of these countries *Campylobacter* spp. has become the most frequently reported cause of bacterial gastrointestinal illness (EC, 2002; FDA, 2000; Friedman *et al.*, 2000; WHO, 2000). The true rate of infection is considered to be considerable higher than the number of reported cases (from 7.6 up to 100 times as high) as many cases never see a physician, not all cases visiting a physician are sampled, and not all positive cases are reported throughout the system (Skirrow 1991; Kapperud 1994; Wheeler *et al.*, 1999; Mead *et al.*, 1999). The incubation period for campylobacteriosis is one day to one week, and infections usually result in mild to moderate symptoms including diarrhoea (frequently with blood in the faeces), abdominal pain, fever, headache, nausea and/or vomiting (WHO, 2000). Symptoms, which are usually self-limiting, may last one day to one week, and in up to 20% of cases, illness lasts for more than a week. More invasive disease such as systemic infections occur in less than 1% of patients with *C. jejuni* infections and are more common in the elderly or very young individuals.

Rare manifestations of *C. jejuni* infections include meningitis, endocarditis and septic abortion. Persons with immunoglobulin deficiencies may show prolonged, severe, and recurrent infections. Campylobacteriosis has been associated with chronic sequelae that include reactive arthritis, inflammation of the liver and kidney, and Guillain-Barré syndrome, a neurological disorder that may result in a reversible paralysis (WHO, 2000; FDA, 2001;). A fatal outcome is rare and is usually confined to very young or elderly patients, or the immuno-compromised suffering from an invasive infection (Schønheyder *et al.,* 1995; Pigrau *et al.,* 1997; WHO, 2000; Oldfield and Wallace 2001). USA estimates 99 deaths linked to foodborne campylobacteriosis annually. This would comprise 5.5% of the total estimated deaths due to foodborne pathogens.

The high incidence of *Campylobacter* spp. diarrhoea as well as its duration and possible sequelae, makes campylobacteriosis highly important from a socio-economic perspective (WHO, 2000). New data from Denmark suggest that the mortality of *Campylobacter* spp. infections is underestimated, and confirms that *Campylobacter* spp. infections may be associated with serious late onset complications (K. Mølbak, pers. comm.).

Antimicrobial treatment is usually not indicated for enteritis of moderate severity. In patients who have moderate-to-severe dysentery (diarrhoea with blood), who are elderly, who are presumed to be bacteraemic with chills and systemic symptoms, or who are at increased risk of complications such as immuno-compromised patients, patients with underlying disease, or pregnant women, an antimicrobial treatment may be of significant benefit (Schønheyder *et al.*, 1995; Pigrau *et al.*, 1997; Oldfield and Wallace, 2001). Thus, it remains essential to be able to treat *Campylobacter* spp. infections with an effective antimicrobial. Erythromycin, tetracyclines and fluoroquinolones are common choices if antimicrobial treatment of campylobacteriosis if needed.

Treatment of serious susceptible enteric infections with an effective fluoroquinolone can reduce the duration of illness and most likely prevent complications and adverse outcomes, including hospitalisation (FDA 2000). Effective treatment of campylobacteriosis has been shown to decrease the duration of illness from 10 days to 5 days and the mean duration of diarrhoea from 5 to 13 days (FDA, 2000).

Data suggest that infections with fluoroquinolone resistant *Campylobacter* spp. are associated with an increased morbidity as compared to infections with sensitive strains (Nachamkin *et al.*, 2002; K. Mølbak pers. comm.). Current data do not indicate that antimicrobial resistant foodborne pathogens carry more virulence factors compared to their non-resistant counterparts. However, unpublished data from Denmark suggest that the detrimental effects of fluoroquinolone resistance in *Campylobacter* spp. is not limited to an increased duration of disease, but that there is an increased risk of intestinal and extra-intestinal complications, and possibly also an increased mortality. However, the difference in mortality between patients infected with fluoroquinolone resistant and sensitive strains was not statistically significant, and a larger data set is needed to determine excess mortality with a greater degree of precision (K. Mølbak, pers. comm.).

The FDA risk assessment (FDA, 2001) estimated that in 1999 one in 32,912 (CI 17,792-52,166) US citizens and one in 17 (CI 12-24) US citizens with campylobacteriosis seeking care and prescribed fluoroquinolones, were affected by fluoroquinolone resistant *Campylobacter* spp.. This represents a probability of 0.0034% (CI 0.0019-0.0056%) for a random US citizen and 6.15% (CI 4.24-8.28%) for a random US citizen with campylobacteriosis seeking care and having prescribed fluoroquinolones, being affected by fluoroquinolone resistant *Campylobacter* spp. The magnitude of the risk may be viewed differently depending on an individual's personal circumstances. For the average citizen, the risk may well be perceived as being very small, whereas for individuals with reduced immunity, who may be more likely seek medical help, the risk may be perceived as significant.

8. VETERINARY MEASURES TO REDUCE THE BURDEN OF FLUOROQUINOLONE RESISTANT *SALMONELLA* SPP. AND *CAMPYLOBACTER* SPP. IN THE FOOD CHAIN

There are two principal approaches for reducing the burden of fluoroquinolone resistant *Salmonella* spp. and *Campylobacter* spp. in the food chain. One approach is to reduce the occurrence of *Salmonella* spp. and *Campylobacter* spp. as such. When the occurrence of the pathogens is reduced, transmission of fluoroquinolone resistant pathogens will automatically be reduced. Another approach is to implement strategies that will diminish the chance for development of fluoroquinolone resistance in *Salmonella* spp. and *Campylobacter* spp. It has to be emphasised that these two strategies do not exclude each other but should be approached in parallel.

8.1. Measures to reduce the prevalence of *Salmonella* spp. and *Campylobacter* spp. in the food chain

8.1.1. Good agricultural practices and Hazard Analysis and Critical Control points (HACCP) procedures

The current use of the term *from the farm to table* clearly identifies the farm as an important part in the production chain, determining the prevalence of zoonotic pathogens such as *Salmonella* spp. and *Campylobacter* spp. in animals and subsequently in animal derived foods. The interrelations between good farming practice (good agricultural practice) and the health status of animals is well established (Aalund *et al.*, 1976; Bandick *et al.*, 1997), and recent studies on UK pig farms have confirmed that simple managerial efforts towards appropriate stocking density and good air quality (appropriate ventilation systems) can reduce the prevalence of diseases and improve disease management (J. Robertson, *pers. comm.*). In turn, major factors contributing to disease prevalence are farm design, animal flow to and between farms, the presence of isolation facilities, animal husbandry practices, and the control of disease vectors (among others rodents and birds, but also workers and visitors to the farm).

Good agricultural practice (GAP) therefore comprises a biosecurity program, including the identification of risk factors for infections and the implementation of hygienic barriers. For example, in poultry production, hygienic measures include 'all-in all-out' management, regular cleaning and

disinfecting of houses and their surroundings between batches of animals, separate equipment per animal house, regular cleaning of feed and water supplies, and regular control of disease vectors. Risk factors in disease transmission are newly introduced animals with unknown disease history, contaminated food and water supplies, and improper disease management or gaps in setting hygienic barriers between different groups of animals at the same farm (including disease transmission by farm personnel and animal health professionals).

Recognition of these risk factors has resulted in the design of HACCP protocols on modern farms in which hazards at each process step are listed and checked for severity and quantitative impact (principle 1). Subsequently, CCPs for each hazard have to be identified and a decision tree presented (principle 2). This implies that for all CCPs target (and or tolerance) levels need to be defined (principle 3), and compliance to these levels needs to be monitored (principle 4). In cases of non-compliance, corrective actions should be taken (principle 5) and their efficiency monitored (principle 6). Finally, the entire production process should be documented with the aim to allow certification of the process and to provide guarantees of product quality (Noordhuizen and Welpelo, 1996; Mitchell, 1998).

During transport and slaughter the same systematic approach towards identification of hazards, and subsequent in-process controls needs to be implemented (Corry *et al.*, 2002). Improved hygiene management during transport of broilers and in slaughterhouses has been shown to significantly reduce the risk of horizontal transmission of *Salmonella* spp. and, in turn, contamination of poultry meat (Heyndrickx *et al.*, 2002).

The risk factors linked to *Salmonella* spp and *Campylobacter* spp. in the entire food production chain, from farm to fork, are presented in the SCVPH opinion on "Foodborne zoonosis" (SCVPH, 2000). Further reference is made to commission decision of 8 June 2001, laying down the rules for regular checks on the general hygiene to be carried out by the operators in establishments according to Directive 64/433/EEC on health conditions for the production and marketing of fresh meat, and in Directive 71/118/EEC on health problems affecting the production and placing on the market of fresh poultry meat (2001/471/EC).

8.1.2. Control of Salmonella *spp. and* Campylobacter *spp. in primary production*

The control of foodborne salmonellosis or campylobacteriosis in primary production is based essentially on the prevention of the introduction of *Salmonella* spp. in the farm, or by reducing the prevalence of *Salmonella* spp. – carriers on infected farms.

Specific intervention methods on the farm can reduce the incidence of *Salmonella* spp and *Campylobacter* spp., especially in poultry. These control options are more feasible if animals are kept indoors during the entire production period. However, this restriction might conflict demands of the society to improve animal welfare. Admittance to the outdoor environment

will reduce the likelihood to obtain *Campylobacter* spp. free herds considering the wide distribution of *Campylobacter* spp. in the environment.

8.1.2.1. Reducing the prevalence of *Salmonella* spp. and *Campylobacter* spp. in the contaminated farms

For highly contaminated flocks, traditionally measures based on improved hygiene and management (all-in, all-out management, cleaning, disinfections, rodent control, hygiene of personnel, control of carriers, and appropriate diagnostic measures) diseased animals with clinical samples from diseased animals or samples taken at necropsy sent for laboratory examination) are known to be efficient. A remaining risk factor, however, are apparently healthy carrier animals, which may be shedding *Salmonella* into the farm environment, or become shedders following handling and transport thus posing a risk for cross contamination. In addition, reduction on infection transmission, particularly in poultry flocks, can be achieved by measures like competitive exclusion and vaccination.

Competitive exclusion: Oral administration of commercial preparations of the intestinal microflora or anaerobic cultures of gut microorganisms from mature, *Salmonella*-free birds, early after hatching to young chicks can prevent intestinal colonisation of *Salmonella* spp.. This method offers the possibility for exclusion of human-pathogenic *C. jejuni* in poultry, as well (Chen and Stern, 2001).

Vaccination: Vaccines against salmonellosis has been tested in poultry to prevent infection, colonisation and shedding of salmonellae. Vaccinal immunity appears to be serovar-specific, but it is possible, for example, to achieve cross-protection against *S.* Enteritidis by a *S.* Gallinarum vaccine. Another possibility is the vaccination with a *Salmonella* DNA-adenine methylase mutant, an attenuated strain conferring cross-protection, presumably via competitive exclusion mechanisms preventing super infections (Dueger *et al.*, 2003).

8.1.2.2. Monitoring programs to obtain *Salmonella*-free farms

S. Enteritidis and *S*. Typhimurium are the most prominent serovars in human foodborne salmonellosis, and the main sources are poultry products, followed by other meat products (pork in particular). In poultry, this implies that appropriate measures have to start from the egg (in breeder herds) and have to be conducted during the entire production phase of the animal. Strict monitoring programs need to be implemented to avoid horizontal and vertical transmission of *Salmonella* spp., especially *S.* Enteritidis with eggs (laying herds), with monthly bacterial testing of faecal samples per house, and bi-monthly testing of layers and broiler parent flocks. These monitoring programs have been shown to reduce the incidence of *Salmonella* spp.-contaminated flocks in many countries. Other programs for the control of *Salmonella* spp. in breeding poultry are to be approved in 2003 in Austria, Denmark, France, Ireland and the Netherlands (Commission Decision

2002/944/EC[1]) with the destruction of infected breeding poultry, and the destruction of incubated hatching eggs originating from contaminated farms.

8.1.3. *Control of* Salmonella *spp. and* Campylobacter *spp. in food*

Despite the above-mentioned control measures during the production phase, animals may carry and/or excrete *Salmonella* spp. and *Campylobacter* spp. at slaughter resulting in the contamination of carcases. A number of measures in the slaughter plant have been suggested to reduce the possibility of carcases (cross)contamination.

Good hygienic practices at slaughter will reduce the rate of contamination of carcasses by faeces, but can not guarantee the absence of *Campylobacter* spp and *Salmonella* spp in meat and meat products. Education of personnel in hygienic handling of foods, particularly raw meat, is essential to keep microbiological contamination to a minimum. The only effective method to eliminate bacterial pathogens entirely from contaminated foods is the application of bactericidal treatments, such as heating (e.g. cooking or pasteurisation) or irradiation.

Decontamination of carcasses by sanitizing agents or irradiation has been considered as well. The prerequisites for the application of sanitizing agents have been presented in the SCVPH opinion on benefits and limitations of antimicrobial treatment for poultry carcasses of 30 October 1998 (SCVPH 1998) and as recently considered by the SCVPH (2003b).

However, it should be emphasised that the use of a **dry chilling** avoids the cross-contamination associated with water-cooling with or without the addition of ice.

Irradiation has been applied to certain foodstuffs which may be contaminated with *Salmonella* spp. (chicken meat, eggs) and which are intended for the direct use by consumers (Communication from the Commission to the European Parliament and the Council on foods and food ingredients authorized for treatment with ionizing radiation in the Community, Brussels, 8.8.2001, COM (2001) 472).

8.2. Measures to reduce the occurrence of fluoroquinolone resistance in *Salmonella* spp. and *Campylobacter* spp. in animals

Resistance to fluoroquinolones is related to their use in animal therapy. Therefore, the availability and the actual use (frequency and dose regime) by professionals influence the prevalence of resistant (zoonotic) bacteria in an animal population.

8.2.1. Veterinary prescriptions

The distribution of veterinary medicinal product is regulated at a different level in individual Member States. Whilst some countries, allow distribution

[1] OJ N° L 326 of 3/12/2002, p. 12

only by pharmacists (DK, S, N, B), other member states (D, NL, F, E, I, G, A) permit veterinarians to obtain (from pharmaceutical industries), store and distribute veterinary medicinal products via their individual veterinary practice. Furthermore, Member States may decide on distribution formalities at the national level, explaining the variations in the level of mandatory prescription for antimicrobials (general use within herd health programmes, use by veterinarians only, use on prescription only).

Implementing the principles of Good Clinical Practice (GCP), antimicrobials should be used only on the basis of a clinical diagnosis, in individual animals or in a distinct group of animals. GCP addresses also the hierarchy in the use of antimicrobials, suggesting the use of conservative antimicrobials in flock medication, and restricting the use of potent antimicrobials to clinical outbreaks on disease. GCP has thus endorsed the principle of prudent use, but the level of implementation varies in the individual EU Member States.

Although not fully implemented, the veterinary profession has expressed their intention to prescribe fluoroquinolones as "third choice antimicrobials". They should be used only in severe clinical cases where according to the professional knowledge and experience small spectrum antimicrobials cannot achieve the therapeutic demands. This concept has been implemented in some EU member states but not in all and is an essential part of GCP. Subsequently, fluoroquinolones should be used prudently as Prescription Only Medicines (POM).

8.2.2. Prudent use and Good Veterinary Practices (GVP)

The Code of Good Veterinary Practice (GVP) refers to a professional code amongst veterinarians. Having been developed as guidance for practitioners, and describing the selection of drugs on the basis of a proper diagnosis, pharmacological and therapeutic considerations and in compliance to existing legislation, GVP has recently defined by FVE as a general Code for the Veterinary Profession (FVE-2002). GVP defines the rules for veterinary prescriptions by demanding records for all medications, providing animal identification, diagnosis and diagnostic measures (testing for sensitivity, antibiograms) and details of the delivered drug. These records will certainly improve the transparency in the use of antimicrobials in veterinary practice and contribute to monitoring and surveillance programs directed to the prevalence of resistant foodborne pathogens.

In more detail, veterinarians need to commit themselves to the following Code of Practice:

-Prescriptions for antimicrobials should be issued only for “animals under the veterinarians care”.

-The choice of the prescribed antimicrobial has to follow the principles of good clinical practice, and an evidence based medicine approach with a proper diagnosis, an estimate (and later confirmation) of the sensitivity of the causal organism(s) should be followed, in consideration of the patient’s anamnesis (immune status, location of infection, duration of disease).

-Dose regimes in terms of individual dosages, dosing intervals and duration of therapy need to be clearly defined and communicated to the patient's owner (including withholding times).

-Therapeutic interventions should be evaluated in terms of efficacy and in consideration of supportive measures such as improvement of farm management, vaccination programs etc.

- Proper management on the farm (production unit) should aim to avoid the need to react to problems with antimicrobial therapy. Proper management includes the use of disease prevention and intervention protocols, based on HACCP principles and developed for the individual farm. The final result will be a reduction of the overall use of antimicrobials.

-Complete records of disease history, intervention programs, drug usage, and incidence and prevalence of antimicrobial resistance should be present at all farms.

-Protocols for the use, storage and disposal of antimicrobials (including batches of feed or drinking water fortified with antimicrobials) should be present at all farms.

Regular campaigns to remind animal owners (including owners of companion animals) of their obligations to comply with prudent-use guidelines for antimicrobials should assist these measures.

8.2.3. Appropriate dose regimes and post-marketing surveillance

With the increasing understanding of the differences in the therapeutic use of antimicrobials exhibiting a time dependent effect, versus antimicrobials such as aminoglycosides and fluoroquinolones exerting predominantly a dose-dependent effect, various attempts have been made to match bacterial sensitivity (dynamics) with pharmacokinetic data obtained from target animal species. Regarding pharmacokinetics, the improved understanding of the role of transport proteins in tissue distribution contributes to the improvement of effective dose regimes (Naruhashi *et al.*, 2001; Yamaguchi *et al.*, 2002). The implementation of PK-PD modelling and populations kinetics, will provide a further instrument to optimise dose regimens for antimicrobials in veterinary therapy, while at the same time reducing the total amount of antimicrobials and the risk for the emergence of resistance (Lees and AliAbadi, 2002; Bousquet-Melou *et al.*, 2002; AliAbadi and Lees, 2000).

Compulsory post-marketing monitoring of the prevalence of resistance under field conditions, will allow the evaluation of the appropriateness of these dose regimes.

8.2.4. Implementation of compulsory resistance monitoring

A proposal for a directive of the European Parliament and of the Council on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC, includes mandatory monitoring of antimicrobial resistance in food animals and foods

thereof. This approach will identify trends and at the same time relevant information on the emergence of antimicrobial resistance in zoonotic agents is obtained. Monitoring should be complementary to the monitoring of human isolates, conducted according to Council Decision 2119/98/EC. General and specific requirements for the monitoring are given in these directives. The monitoring shall include at least antibiograms for a representative number of isolates of *Salmonella* spp., *Campylobacter jejuni* and *C. coli* from cattle, pigs and poultry (http://europa.eu.int/eur-lex/en/com/pdf/2001/en_501PC0452_01. pdf).

9. 'PROS' AND 'CONS' OF THE VETERINARY USE OF FLUOROQUINOLONES – RISK BENEFIT ANALYSIS

Fluoroquinolones are important antimicrobials in veterinary medicine due to their specific pharmacokinetic and pharmacodynamic properties, allowing the successful treatment of specific infections in many cases where other antimicrobials are less successful due to limited ability to penetrate tissue barriers and to reach therapeutic levels at target. Moreover, fluoroquinolones are used in those cases in which multi-resistance to other (conventional) antimicrobials (including sulfanomides, tetracyclines and broad spectrum penicillines) requires the use of a drug, which is not impaired in its efficacy by these forms of (transmissible) drug resistance. The prevalence of multi-drug resistance varies between farms, regions and countries and is related to the overall use (or misuse) of antimicrobials at the farm level. Fluoroquinolones are especially effective against infections caused by Gram-negative bacteria. These infections, including among others, Salmonellosis, enteropathogenic *E. coli*, pasteurellosis and mycoplasma infections, are common with high morbidity and potentially high mortality. Thus, following the demand of optimal animal health care, fluoroquinolones are important alternatives in the treatment of these diseases in many animal species, in those cases, were conventional antimicrobials cannot be applied due to the above-mentioned limitation.

However, the use of fluoroquinolones in veterinary medicine, and particularly in poultry, comprises the risk of selection for fluoroquinolone-resistant bacteria. Via direct contact of via the food chain, these bacteria might reach human beings, thus contributing to the overall risk for bacterial resistance in the human population. This applies particular to *Salmonella* spp. and *Campylobacter* spp. isolated from poultry or poultry meat, which have been found to be implemented in many cases of acquired fluoroquinolone resistance in humans. Moreover, scientific data from several countries (UK, USA, NL) demonstrated that the usage of fluoroquinolones in food animals is linked to an increased incidence of fluoroquinolone resistant *Campylobacter* spp. infections.

In most cases, *Campylobacter* spp. infections in humans do not require antimicrobial treatment, and thus acquired fluoroquinolone-resistance may be of little consequence from a clinical perspective. However, in patients who have moderate-to-severe dysentery (diarrhoea with blood), who are elderly, who are presumed to be bacteraemic with chills and systemic symptoms, or who are at increased risk of complications such as immuno-compromised patients, patients with underlying disease, or pregnant women, an antimicrobial treatment may be of significant benefit. In these populations, where antimicrobial treatment is needed or

recommended to combat foodborne diarrhoea (young, elderly and immuno-compromised) effective alternatives such as erythromycin are available (although an increase in the resistant to this antimicrobial is recorded as well). This again emphasises the responsibility of the entire medical profession (human and veterinary medicine) to endorse the principles of prudent use of antimicrobials. Moreover, optimal dose-regimes need to be implemented also in human (as in veterinary) medicine as essential measure to reduce the risk for acquired resistance.

As a precaution, veterinary measures should be taken, to avoid or reduce the prevalence of fluoroquinolone resistance in livestock, and particularly in poultry. These measures, as mentioned above, should include the following items:

- Setting standards for the use of fluoroquinolones in veterinary practice, by demanding their selective use by-veterinarians-only in those cases were the therapeutic goal is not achievable with other therapeutic agents.

- Evaluation of the dose-regimes for fluoroquinolones in the light of the recent understanding of the pharmacodynamics and pharmacokinetics of these drugs to reduce the likelihood of resistance development.

- Mandatory monitoring the use of antimicrobials in different categories of animals (species- and age-groups, particularly poultry flocks) to gain insight in the actual use of antimicrobial therapy, subsequently allowing corrective measures if the applications appear to be non-justified.

- Mandatory resistance monitoring in all EU Member States, to analyse trends in resistance development and to allow immediate measures in cases of non-compliance to the above mentioned criteria for use.

As yet, any quantitative risk assessment to estimate the contribution of the use of quinolones in veterinary medicine to the overall prevalence of quinolone resistance in the human population is hampered by the large variation in resistance prevalence between countries, seasonal fluctuations of campylobacteriosis, incompleteness of epidemiological data, and considerable difference in the attitude towards prescription of fluoroquinolones in human therapy. Moreover, even in EC, sampling, culture techniques and test methods have not been harmonized yet, presenting a further obstacle in quantitative risk assessment.

Human infections seem to be acquired in many cases during travelling (EC, 2002), or result from consumption of contaminated (non-EU imported) poultry. This implies that the approach of a prohibition of the use of fluoroquinolones in poultry production alone, will be insufficient and only a global management of fluoroquinolones will significantly reduce the risk for fluoroquinolone resistance.

10. CONCLUSIONS

- There is accumulating and convincing evidence of the development of fluoroquinolone resistance in *Salmonella* spp. and *Campylobacter* spp. following the introduction of fluoroquinolones into regional markets. Moreover, an association between the use of fluoroquinolones in animals and the incidence of fluoroquinolone-resistant *Salmonella* spp. and *Campylobacter* spp. in humans has been documented.

- An increasing prevalence of fluoroquinolone resistant *Salmonella* spp. and *Campylobacter* spp. in the human environment can influence human therapy. In turn, reducing the use of fluoroquinolones in all sectors will contribute to the reduction of the risk for acquired fluoroquinolone resistance in humans.

- Due to the importance of fluoroquinolones in human and animal therapy, a *prudent use* of fluoroquinolones is essential as preventive measure

- In the EU, *Salmonella* spp. and *Campylobacter* spp. infections are predominantly foodborne (poultry being the main source for *Campylobacter* spp. infections) whereas salmonellosis is multifactorial. Transmission of resistant *Salmonella* spp. and *Campylobacter* spp. by other routes, including direct and indirect contact with animals, have been recognized. The control of *Salmonella* spp. in the primary animal production has been shown to be beneficial in terms of reducing human risk to acquire fluoroquinolone resistance.

- A prominent part of the *prudent-antimicrobial-use* concept is the surveillance of fluoroquinolone resistant *Salmonella* spp. and *Campylobacter* spp., and the surveillance of the total usage of fluoroquinolones stratified into animal species and indications.

- At present, there is a lack of specified *prudent-use* guidelines, defining the appropriate and responsible use of fluoroquinolones. The concept of *prudent use* is a global responsibility, as due to global trade and increasing human mobility, the use of fluoroquinolones in humans or animals in one country will affect the level of resistance in another.

11. RECOMMENDATIONS

To reduce the risk for foodborne infections and the transfer of fluoroquinolone resistant *Salmonella* spp. and *Campylobacter* spp. from animal(s) to man, precautionary veterinary measures should be directed towards:

(I) a reduction of the prevalence of *Salmonella* spp. and *Campylobacter* spp. in food producing animals by implementing strict hygiene controls at the farm, during slaughter and transport as well as at the retail and even consumer's level.

(II) a reduction of the overall use of fluoroquinolones by restricting their application to the selective use upon prescription of a licensed veterinarian.

A code of practice for the *prudent-use* of fluoroquinolones needs to be established in human and animal medicine, addressing the the following aspects:

- Fluoroquinolones have to be categorized as POMs (prescription-only-medicines) to be used only under the control of a physician/veterinarian.

- The application of fluoroquinolones is justified only if the clinical need/indication is based on appropriate diagnostic measures.

(III) the establishing of a mandatory antimicrobial resistance monitoring in the EU

(IV) the type and distribution of information to all stakeholders should be reviewed to improve risk communication, including the definition of *prudent-use*.

(V) the public at large should be informed adequately about the risk of transfer of fluoroquinolone-resistant *Salmonella* spp. and *Campylobacter* spp. from animals (including pet animals) to man, and via the food chain.

12. REFERENCES

Aalund, O., Willeberg, P., Mandrup, M., Riemann, H., (1976). Lung lesions at Slaughter: Association to Factors in the Pig Herd. Nord. Vet. Med., 28, 487-495.

ACMSF (Advisory Committee on Microbiological Safety of Foods (UK)), (2001). Second Report on *Salmonella* in Eggs. The Stationery Office, London. ISBN 0-11-322466-4.

ACMSF (Advisory Committee on Microbiological Safety of Foods (UK)), (1998) Report on Microbial Antibiotic Resistance in Relation to Food Safety. The Stationary Office, London. ISBN 0-11-322283-1.

AliAbadi, S., Lees, P., (2000). Antibiotic treatment for animals: effect on bacterial population and dosage regimen optimisation. Int. J. Antimicrob. Agents, 14, 307-313.

Bandick, N., Dahms, S., Kobe, A., Tittmann, A., Weiss, H., Fries, R., (1997). Zusammenhänge zwischen Mastbedingungen und Ergebnissen der amtlichen Fleischuntersuchung. Proc. Deutsche Veterinärmedizinische Gesellschaft, 38. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene in Garmisch-Partenkirchen, 29.9-2.10.1999, Vol. I, pp. 279-285.

Barza, M., Travers, K., (2002). Excess infections due to antimicrobial resistance: the "Attributable Fraction". Clin. Infect. Dis., 34 Suppl 3, 126-130.

Blackmore, C.G., McNaughton, O.A., van Veen, H.V., (2001). Multi-drug transporters in prokaryotic and eukaryotic cells: physiological functions and transport mechanisms. Mol. Membr. Biol., 18, 97-103.

Bousquet-Melou, A., Bernard, S., Schneider, M., Toutain, P.L., (2002). Pharmacokinetics of marbofloxacin in horses. Equine Vet. J., 34, 360-365.

CCFH (Codex Committee on Food Hygiene) (2000). Risk profile on antimicrobial-resistant bacteria in food. Discussion paper to the 33rd Session, Washington DC.

Chen, H.C., Stern, N.J., (2001). Competitive exclusion of heterologous *Campylobacter* spp. in chicks. Appl. Environ. Microbiol., 2001, 67,848-851.

Cohen, M.L., (1992). Epidemiology of drug resistance : Implications for a post-antimicrobial era. Science, 234, 964-969.

Cohen, S.P., Levy, S.B., Foulds, J., Rosner, J.L., (1993). Salicylate induction of antibiotic resistance in E. coli: activation of the mar operon and a mar-independent pathway. J. Bacteriol., 175, 7856-7862.

Corry, J.E., Allen, V.M., Hudson, W.R., Breslin, M.F., Davies, R.H., (2002). Sources of Salmonella on broiler carcasses during transportation and processing : modes of contamination and methods of control. J. Appl. Microbiol., 2002, 92, 424-432.

Critchley, I.A., Blosser-Middleton, R.S., Jones, M.E., Karlowsky, J.A., Karginova, E.A., Thornsberry, C., Sahm, D.F., (2002). Phenotypic and genotypic analysis of levofloxacin-resistant clinical isolates of *Streptococcus pneumoniae* collected in 13 countries during 1999-2000. International Journal of Antimicrobial Agents, 20, 100-107.

D'Aoust, J.-Y., Warburton, D.W., Sewell, A.M., (1985). *Salmonella typhimurium* phage type 10 from cheddar cheese implicated in a major Canadian foodborne outbreaks. J. Food Protect., 48, 1062-1066.

DANMAP, 2001 (2002). Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. ISSN 1600-2032. (www.vetinst.dk).

Deguchi, T., Fukuoka, A., Yasuda, M., Nakano, M., Ozeki, S., Kanematdu, E., Nishino. S, Ban Y., Kawaka, Y., (1997). Alterations in the GyrAsubunit of DNA gyrase and the ParC subunit of topoisomerase IV in quinolone-resistant clinical isolates of Klebsiella pneumoniae. Antimicrob. Agents Chemother., 41, 699-701.

Drusano, G.L., Preston, S.L., Gotfried, M.H., Danziger, L.H., Rodvold, L.A., (2002). Levofloxacin penetration into epithelial lining fluid as determined by population pharmacokinetic modelling and Monte Carlo simulation. Antimicrob. Agents Chemother., 46, 568-589.

Dueger, E.L., House, J.K., Heithof, D.M., Mahan, M.J., (2003). Salmonella DNA-adenine-methylase mutant prevent colonization of newly hatched chickens by homologous and heterologous serovars. Int. J. Food Microbiol., 80, 153-159.

Dunne, E.F., Fey, P.D., Kludt, P., Reporter, R., Mostashari, F., Shillam, P., Wicklund, J., Miller, C., Holland, B., Starney, K., Barrett, T.J., Rasheed, J.K., Tenover, F.C., Ribot, E.M., Angulo, F.J., (2000). Emergence of Domestically Acquired Ceftriaxone-Resistant *Salmonella* Infections Associated With AmpC β-Lactamase. JAMA, 284, 3151-3156.

EMEA (The European Agency for the Evaluation of Medical Products), (1999). Antibiotic resistance in the European Union associated with therapeutic use of veterinary medicines. Report and qualitative risk assessment by the committee for veterinary medicinal products. (EMEA/CVMP/342/99-Final) 14 July 1999. (http:/www.emea.eu.int)

EMEA (The European Agency for the Evaluation of Medical Products), (2001a). Guideline on Pre-authorisation studies to Assess the Potential Resistance Resulting from the use of Antimicrobial Medical Products (EMEA/CVMP/244/01-FINAL). (http://www.emea.eu.int/pdfs/vet/swp/024401en.pdf)

EMEA (The European Agency for the Evaluation of Medical Products), (2001b). Revised guideline on Antimicrobials for Generals Veterinary Use in Target Animal Species (EMEA/CVMP/627/01-FINAL). (http://www.emea.eu.int/pdfs/vet/ewp/062701en.pdf)

EMEA (The European Agency for the Evaluation of Medical Products), (2001c). Guideline on the SPC for Antimicrobial Products (EMEA/CVMP/612/01-FINAL). (htpp://www.emea.eu.int/pdfs/vet/ewp/061201en.pdf)

Endtz, H.P., Ruijs, G.J., van Klingeren, B., Jansen, W.H., van der Reyden, T., Mouton, R.P., (1991). Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J. Antimicr. Chemother., 27, 199-208.

EC (European Commission), (2002). Trends and Sources of Zoonotic Agents in Animals, Feedstuffs, Food and Man in the European Union and Norway in 2000 to the European Commission in accordance with Article 5 of the Directive 92/117/EEC, prepared by the Community Reference Laboratory on the Epidemiology of Zoonoses, BgVV, Berlin, Germany. Working document SANCO/927/2002, Part 1.

EC (European Commission), (2003). Trends and Sources of Zoonotic Agents in Animals, Feedstuffs, Food and Man in the European Union and Norway in 2000to the European Commission in accordance with Article 5 of the Directive 92/117/EEC, prepared by the Community Reference Laboratory on the Epidemiology of Zoonoses, BgVV, Berlin, Germany.

Everett, M.J.M., Jin, Y.F., Ricci, V., Piddock, L.J.V., (1996). Contribution of individual mechanisms to fluroquinolone resistance in 36 Escherichia coli strains isolated from humans and animals. Antimicrob. Agents Chemother., 40, 2380-2386.

Everett, M.J., Piddock, L.J.V. (1998). Mechanisms of resistance to fluoroquinolones. Quinolone Antibacterials In: Kuhlmann, J., Dalhoff, A., zZeiler, H.-J., (eds), Springer Verlag, New York, 1998, 259-296.

FAO/WHO Collaborating Centre for Research and Training in Food Hygiene and Zoonoses, (2001). WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe, Seventh Report 1993-1998 (eds K Schmidt and C Tirado), Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), Berlin, ISBN 3-931675-70-X, ISSN 0948-0307.

FAO/WHO, (2002). Report of the joint FAO/WHO expert consultation on risk assessment of *Campylobacter* spp. in broiler chickens and *Vibrio* spp. in seafood. Bangkok, Thailand, August 5-9, 2002.

FDA (Food and Drug Administration), (2000). Enrofloxacin for poultry; Opportunity for hearing. Federal Register 65, 211, October 31.

FDA (Food and Drug Administration), (2001). Risk assessment on the human health impact of fluoroquinolone resistant *Campylobacter* associated with the consumption of chicken, October 18 2000, revised January 5 2001.

Fey, P.D., Safranek, T.J., Rupp, M.E., Dunne, E.F., Ribot, E., Iwen, P.C., Bradford, P.A., Angulo, F.J., Hinrichs, S.H., (2000). Ceftriaxone-Resistant Salmonella Infection Acquired by a Child from Cattle. NEJM, 342, 1242-1249.

Friedman, C.R., Neimann, J., Wegener, H.C., Tauxe, R.V., (2000). Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin I, MJ Blaser. *Campylobacter* 2nd edition. ASM press, Washington DC, 121-138.

Frimodt-Møller, N., (2002). How predictive is PK/PD for antibacterial agents. Int. J. Antimicrob. Agents, 19, 333-339.

FVE (Federation Veterinarian European), 2002. FVE Newsletter, October 2002.

Fung-Tomc J., Gradelski, E., Huscko, E., Minassian, B., Bonner, D.P., (2001). Activity of gatifloxacin against strains resistant to ofloxacin and ciprofloxacin and its ability to select for less susceptible bacterial variants. Int. J. Antimicrobial Agents, 18, 77-80.

Fung-Tomc, J.C., Gradelski, E., Valera, L., Kolek, B., Bronner, D.P., (2000). Comparative killing rates if fluoroquinolones and cell wall-active agents. Antimicrob. Agents Chemother., 44, 1377-1380.

Glynn, M.K., Reddy, V., Hutwagner, L., Rabatsky-Het, T., Shiferaw, B., Vugia, D.J. (2003). Prior antimicrobial agent use increases the risk of sporadic infections with multidrug-resistant *Salmonella enterica* serotype Typhimurium: a FoodNet case-control study. CID, 2003 *in press.*

Goldman, J.D., White, D.G., Levy, S.B., (1996). Multiple antibiotic resistance (MAR) locus protects *E. coli* from rapid killing by fluoroquinolones. A. A. C., 40, 1266-1269.

Griggs, D.J., Gensberg, K., Piddock, L.J.V., (1996). Mutations in *gyrA* gene of quinolone-resistant salmonella serotypes isolated from humans and animals. Antimicrob. Agents Chemother., 33, 1173-1189.

Grohe, K., (1998). The chemistry of quinolones: methods of synthesising the quinolone ring system. In: Kuhlman, J. *et al.* (eds) Quinolone Antibacterials. Springer-Verlag New York p. 32.

Heisig, P., Kratz, B.M., Halle, E., Graser, Y., Altwegg, M., Rabsch, W., Faber, J.P., (1995). Identification of DNA gyrase A mutations in ciprofloxacin-resistant isolates of *Salmonella typhimurium* from men and cattle in Germany. Microb. Drug Resist, 1,211-218.

Helms, M., Vastrup, P., Gerner-Smidt, P., Mølbak, K., (2002). Excess mortality associated with antmicrobial drug-resistant *Salmonella* Typhimurium. Emerg. Infect. Dis., 8, 490-495.

Heurtin-Le Corre, C., Donnio, P.Y., Perrin, M., Travert, M.F., Avril, J.L., (1999). Increasing incidence and comparison of nalidixic acid-resistant *Salmonella enterica* subsp. *enterica* serotype Typhimurium isolates from humans and animals. J. Clin. Microbiol., 37, 266-269.

Heyndrickx, M., Vandekerchove, D., Herman, L., Rolier, I., Grijspeerdt, K. De Zutter, L., (2002). Routes of *Salmonella* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. Epidemiol. Infect., 2002, 129, 253-265.

Holmberg, S.D., Osterholm, M.T., Senger, K.A., Cohen, M.L., (1984a). Drug-resistant salmonella from animals fed antimicrobials N. Engl. J. Med., 311, 617-622.

Holmberg, S.D., Wells, J.G., Cohen, M.L., (1984b). Animal-to-man transmission of antimicrobial-resistant *Salmonella*: Investigations of US outbreaks, 1971-1983. Science, 225, 833-835.

Hopper, D.C., (2002). Fluoroquinolone resistance among Gram-positive cocci. Lancet Infect. Dis., 2, 530-538.

Hyatt, J.M., Mckinnon, P.S., Zimmer, G.S., Schentay, J.J., (1995). The importance of pharmacokinetics/pharmacodynamic surrogate markers to outcome: focus on antimicrobial agents. Clin Pharmacokinet., 28, 143-160.

Hyatt, J.M., Luzier, A.B., Forrest, A., Ballow, C.H., Schentag, J.J., (1997). Modelling the response of pneumonia to antimicrobial therapy. Antimicrob. Agents Chemother. 41, 1269-1274.

ICMSF (International Commission on Microbiological Specifications for Foods), (1996). Microorganisms in Foods 5: Characteristics of Microbial Pathogens. Blackie Academic & Professional, London; Kluwers Academic/Plenum Publishers, New York.

Jacobs-Reitsma, W., (2000). *Campylobacter* in the food supply. In: Nachamkin I, MJ Blaser. *Campylobacter* 2nd edition. ASM press, Washington DC,121-138.

Jones, K., (2001). Campylobacters in water, sewage and the environment. J. Appl. Microbiol., 90, 68S-79S.

Jones, R.N., (2002). Microbiology of newer fluoroquinolones: focus on respiratory pathogens. Diagnostic Microbiology and Infectious Disease, 44, 213-220.

Kaats, G.M., Seo, S.M., (1998). Topoisomerase mutations in fluoroquinolone-resistant and methicillin-susceptible and -resistant clinical isolates of Staphylococcus aureus. Antimicrob. Agents Chemother., 42, 197-201.

Kaatz, G.W., Barriere, S.L., Schaberg, D.R., Fekety, R., (1987). The emergence of resistance to ciprofloxacin during treatment of experimental *Staphylococcus aureus* endocarditis. J. Antimicrob. Chemother., 20, 753-758.

Kapperud, G. (1994). *Campylobacter* infections. Epidemiology, risk factors and preventive measures (in Norwegian). Tidsskr. Nor. Lægeforen. 114, 795-799.

Kramer, J.M., Frost, J.A., Bolton, F., Wareing, D.R.A., (2000). *Campylobacter* contamination of raw meat and poultry at retail sale: Identification of multiple types and comparison with isolates from human infection. J. Food Prot. 63, 1654-1659.

Kruse, H., Skov Simonsen, G. (eds.), (2002). NORM/NORM-VET 2001. Consumption of antimicrobial agents and occurrence of antimicrobial resistance in Norway. ISSN 1502-2307, Tromsø / Oslo.

Kruse, H., Nødtvedt, A., Hofshagen, M., Stavnes, T., Lassen, J., (2002). Prevalence of resistance in *Campylobacter jejuni* from humans in Norway linked to travel abroad. 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC, 42nd), San Diego, California, USA, September 27-30.

Kuhlmann, J., Schaefer, H.-G., Beermann, D., (1998). Clinical Pharmacology. Quinolone Antibacterials In : Kuhlmann, J., Dalhoff, A., Zeiler, H.-J. (eds). Springer Verlag New York, 1998, pp. 339-406.

Lees, P., AliAbadi, F.S., (2000). Rationalising dosage regimens of antimicrobial drugs : a pharmacological perspective. J. med. Microbiol., 49, 943-945.

Lees, P., AliAbadi, F.S.,(2002). Rational dosing of antimicrobial drugs : animals versus humans. Int. . Antimicrob. Agents, 19, 269-284.

Lehmacher, A., Bockemühl, J., Aleksis, S., (1995). A nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. J. Infect. Dis., 115, 501-511.

Lucey, B., Cryan, B., O'Halloran, F., Wall, P.G., Buckley, T., Fanning, S., (2002). Trends in antimicrobial susceptibility among isolates of Campylobacter species in Ireland and the emergence of resisatnce to ciprofloxacin. Vet. Rec., 151, 317-320.

Luo, N., Sahin, O., Lin, J., Michel, LO, Zhang, Q., (2003). In vivo selection of Campylobacter isolates with high levels of fluoroquinolone resistance associated with gyrA mutations and the function of the CmeABC efflux pump. Antimicrob. Agents Chemother., 47, 390-394.

Martínez-Martínez, L., Pascual, A., Jacoby, G.A., (1998). Quinolone resistance from a transferable plasmid. The Lancet, 351, 797-799

Maxwell, A., Critchlow, S.E., (1998). Mode of actions. In: Kuhlman, J., Dalhoff, A., Zeiler, H.-J. (eds) Quinolone Antibacterials. Springer-Verlag New York, pp.119-166.

McCullough, N.B., Eisele, C.W., (1951). Experimental human salmonellosis. J. Inf. Dis., 88, 278-279.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V., (1999). Food-related illness and death in the United States. Emerg. Inf. Dis., 5, 607-625.

Mitchell, R.T., (1998). Why HACCP fails. Food Control 9, 101.

Mølbak, K., Gerner-Smidt, P., Wegener, H.C., (2002). Increasing quinolone resistance in *Salmonella enterica* serotype Enteritidis. Emerg. Infect. Dis., 8, 514-515.

Moore, J.E., Crowe, M., Heaney, N., Crothers, E., (2001). Antibiotic resistance in *Campylobacter* spp. isolated from human faeces (1998-2000) and foods (1997-2000) in Northern Ireland: an update. J. Antimicr. Chemother., 48, 455-457.

MMWR (Morbidity and Mortality Weekly Report), (2002). Outbreak of Multidrug-Resistant *Salmonella* Newport - United States, January-April 2002. MMWR Weekly, June 28, 2002 / 51(25), 545-548.

Morrissey, I., (1997). Bactericidal index: a new way to assess quinolone bactericidal activity in vitro. J. Antimicrob. Therap., 39, 713-717.

Nachamkin, I., Ung, H., Li, M., (2002). Increasing fluoroquinolones resistance in Campylobacter jejuni, Pennsylvania, USA, 1982-2001. Emerg. Infect. Dis., 8, 1501-1503.

Naruhashi, K., Tamai, I., Inonue, N., Muraoka, H., Say, Y., Suzuki, N., Tsuji, A., (2001). Active intestinal secretion of new quinolone antimicrobials and the partial contribution of P-glycoprotein. J. Pharm. Pharmacol., 53, 699-709.

Neu,H.C., (1990). Chemical evolution of the fluoroquinolone antimicrobial agents. Am. J. Med., 87, 2-9.

Noordhuizen, J.P.T.M., Welpelo, H.J., (1996). Sustainable improvement of animal health care by systematic quality risk management according to HACCP concept. Veterinary Quaterly, 18,121-126.

Oldfield, E.C., Wallace, M.R. (2001). The role of antibiotics in the treatment of infectious diarrhea. Gastroenterol. Clin. N. Amer., 30: 817-36.

Pendland, S.L., Neuhauser, M.M., Garey, K.W., Prause, J.L., Jung, R., (2002). Comparative killing rates of gatifloxacin and ciprofloxacin against 14 clinical isolates: impact of bacterial strain and antibiotic concentration. Diagn. Microbiol Infect. Diseases, 44, 59-61.

Petersen, U., Schenke, T., (1998). The chemistry of quinolones. In: Kuhlman, J. (eds) Quinolone Antibacterials. Springer-Verlag New York, p. 63.

Piddock, L.J.V., (1995). Quinolone resistance and *Campylobacter* spp. J. Antimicr. Chemother. 36, 891-898.

Piddok, L.J.V., Ricci, V., Pumbwe, L., Everett, M.J., Griggs, D.J., (2003). Fluoroquinolone resistance in *Campylobacter* species from man and animals: detection of mutations in topoisomerase genes. J. Antimicr. Chemother., 51, 19-26.

Pigrau, C., Bartolome, R., Almirante, B., Planes, AM, Gavalda, J., Pahissa, A., (1997). Bacteremia due to *Campylobacter* species: clinical findings and antimicrobial susceptibility patterns. Clin. Infect. Dis. 6, 1414-20.

Prats, G., Mirelis, B., Llovet, T., Muñoz, C., Miró, E., Navarro, F., (2000). Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona. Antimicrob. Agents Chemother. 44, 1140-1145.

Prescott, J.F., Baggot, J.D., Walker, R.D. (eds), 2000. Antimicrobial theraphy in veterinary medicine. Jowa State University, Press, Ames, Iowa, 2002.

Preston, S.L., Drusano, G.L., Berman, A.L., Fowler, C.L., Chow, A.T., Dornseif, B., Reichl, V., Natarajan, J., Corrado, M., (1998a). Pharmacodynamics in levofloxacin: a new paradigm for early clinical trials. JAMA, 279, 125-129.

Preston, S.L., Drusano, G.L., Berman, A.L., Fowler, C.L., Chow, A.T., Dornseif, B., Reichl, V., Natarajan, J., Corrado, M., (1998b). Lecofloxacin population pharmacokinetics and creation of a demographic model for prediction of individual drug clearance in patients with serious community-acquired infection. Antimicrob. Agents Chemother., 42, 1098-1104.

Reyba, F., Huesca, M., Gonzales,V., Fuchs, L.Y., (1995). *Salmonella typhimurium* gyrA mutations associated with fluoroquinolone resistance. Antimicrob. Agents Chemother., 39: 1621-1623.

Robinson, M.J., Mahin, B.A., Gootz, T.D., McGuirk, P.R., Mognihan, U., Sutcliffe, J.A., Osheroff, N., (1991). Effects of quinolone derivatives on eukaryotic topoisomerase II. Anovel mechanism for enhancement of enzyme mediated DNA cleavage. J. Biol. –Chem., 266, 1585-1592

Sàenz, Y., Zarazago, M., Lantero, M., Gastañares, MJ, Baquero, F., Torres, C., (2000). Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998. Antimicr. Agents Chemother., 44, 267-271.

Schacht, P., (1998). Clinical use of quinolones. Quinolone antibacterials In: Kuhlmann, J., Dalhoff, A., Zeiler, H.-J. (eds). Springer Verlag New York, 1998, 421-453.

Schentag, J.J., Scully, B.E., (1999). Quinolones. In: Yu VL (eds). Antimicrobial Therapy and Vaccines. William and Wilkens, New York., p 875-903.

Schønheyder, H.C., Søgaard, P., Frederiksen, W., (1995). A survey of *Campylobacter* bacteremia in three Danish counties, 1989 to 1994. Scand. J. Infect. Dis., 27, 145-148.

SCVPH (Scientific Committee on Veterinary Measures relating to Public Health), (2003a). Opinion on Salmonellae in Foodstuffs, adopted on April 2003. (http://europa.eu.int/comm/food/fs/sc/scv/outcome_en.html#opinions)

SCVPH (Scientific Committee on Veterinary Measures relating to Public Health), (2003b). Opinion on The evaluation of antimicrobial treatments for poultry carcasses, adopted on April 2003.
(http://europa.eu.int/comm/food/fs/sc/scv/out63_en.pdf)

SCVPH (Scientific Committee on Veterinary Measures relating to Public Health), (2001). Opinion on Foodborne zoonoses, adopted on 12 April 2000. (http://europa.eu.int/comm/food/fs/sc/scv/out32_en.pdf)

SCVPH (Scientific Committee on Veterinary Measures relating to Public Health), 1998. Report on benefits and limitations of antimicrobial treatment for poultry carcasses, adopted on 30 October 1998.
(http://europa.eu.int/comm/food/fs/sc/scv/out14_en.pdf)

SSC (Scientific Steering Committee), (1999). Opinion on antimicrobial resistance, adopted on 28 May 1999. (http://europa.eu.int/comm/food/fs/sc/ssc/out50_en.pdf)

Skirrow, M.B., (1991). Epidemiology of *Campylobacter* enteritis. Int. J Food Microbiol., 12, 9-16.

Smith, K.E., Besser, J.M., Hedberg, C.W., Leano, F.T., Bender, J.B., Wicklund, J.H., (1999). Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. N. Engl. J. Med., 340, 1525-1532.

Spika, J.S., Waterman, S.H., Soo Hoo, G.W., St.Louis, M.E., Pacer, R.E., James, S.M., Bissett, M.L., Mayer, L.W., Chiu, J.Y., Hall, B., Greene, K., Potter, M.E., Cohen, M.L., Blake, P.A., (1987). Chloramphenicol-resistant *Salmonella newport* traced through hamburger to dairy farms. N. Engl. J. Med., 316, 565-570.

Stahlmann, R., Lode, H., (1998). Concentration-effect relationship of the fluoroquinolones. In: Kuhlmann, J., Dalhoff, A., Zeiler, H.-J., (eds), Quinolone Antibacterials, Springer Verlag New York, 1998, pp. 407-420.

Swann, M.M., 1969. Report. Joint Committee on the use of antibiotics in animals husbandry and veterinary medicine. HMSO, London.

Taylor, D.E., Chau, A.S.S., (1997). Cloning and nucleotide sequence of the *gyrA* gene from *Campylobacter fetus* subsp. *fetus* ATCC 27374 and characterization of ciprofloxacin-resistant laboratory and clinical isolates. Antimicrob. Agents Chemother., 41, 655-671.

Threlfall, E.J., Ward, L.R., Rowe, B., (1997). Increasing incidence of resistance to trimethoprim and ciprofloxacin in epidemic *Salmonella typhimurium* DT 104 in England and Wales. Eurosurveillance Monthly, 2, 81-84.

Threlfall, E.J., Frost, J.A., Rowe, B., (1999a). Fluoroquinolone resistance in Salmonellas and Campylobacters from humans. Br. Med. J., 318: 943-944.

Threlfall, E.J., Ward, L.R., Rowe, B., (1999b). Resistance to ciprofloxacin in non-typhoidal salmonellas from humans in England and Wales - the current situation. Clin. Microbiol. Infect., 5: 130-134.

Threlfall, E.J., Fisher, I.S.T., Berghold, C., (2003). Antimicrobial drug resistance in isolates of *Salmonella enterica* from cases of salmonellosis in humans in Europe 2000: results of international multi-centre surveillance. Eurosurveillance Monthly, 8, 41-45.

Tran, J.H., Jacoby, G.A., (2002). Mechanism of plasmid-mediated quinolone resistance. PNAS, 99, 5638-5642.

van Pelt, W., Van der Zee, H., Wannet, W.J.B., Van de Giessen, A.W., Mevius, D.J., Bolder, N.M., Komijn, R.E., Van Duynhoven, Y.T.H.P., (2003). Explosive increase of *Salmonella* Java in poultry in the Netherlands: Consequences for public health. Eurosurveillance Monthly, 8, 31-35.

Velázquez, J.B., Jiménez, A., Chomón, B., Villa, G., (1995). Incidence and transmission of antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli.* Antimicr. Agents Chemother., 32, 1170-1173.

Vila, J., Ruiz, J., Goni, P., De Anta, M.T.J., (1996). Detection of mutations in *parC* in quinolone- resistant clinical isolates of *Escherichia coli*. Antimicrob. Agents Chemother, 40, 491-493.

Wall, P.G., Morgan, D., Lambden, K., Ryan, M., Griffin, M., Threlfall, E.J., (1994). A case control study of infection with an epidemic strain of multiresistant *Salmonella typhimurium* DT104 in England and Wales. Commun. Dis. Rep. CDR Rev., 4, 130-135.

Wheeler, J.G., Sethi, D., Cowden, J.M., Wall. P.G., Rodrigues, L.C., Tompkins, D.S., Hudson, M.J., Roderick, P.J., (1999). Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. British Med. J., 318, 1046-1050.

WHO (World Health Organisation), (1998). Use of quinolones in Food Animals and Potential Impact on Human Health. Report of a World Health Organisation's Meeting, 2-5 June 1998, Geneva, Switzerland. http://www.who.int/emc-documents/antimicrobial_resistance/whoemczoo974c.html

WHO (World Health Organisation), (2000). Fact sheet No. 255, Nov. 2000.

WHO (World Health Organisation), (2001). The Increasing Incidence of Human Campylobacteriosis. Report and Proceedings of a WHO Consultation of Experts, Copenhagen, Denmark, 21-25 November 2000. World Health Organization, Department of Communicable Disease Surveillance and Response. http://www.who.int/emc-documents/zoonoses/whocdscsraph20017c.html

WHO (World Health Organisation), (2002). Monitoring antimicrobial usage in food animals for the protection of human health. Report of a WHO consultation. Oslo, Norway. 10-13 September 2001. http://www.who.int/emc-documents/antimicrobial_resistance/whocdscsreph200211.html

Yamaguchi Hiroaki, Ikuko, Y., Saito, H., Inui, K-I, (2002). Pharmacokinetic role of P-glycoprotein in oral bioavailability and intestinal secretion of grepafloxacin in vivo. JPET, 300, 1063-1069.

Zhanel, G.G., Walkty, A., Nichol, K., Smith, H., Noreddin, A., Hoban, D.J., (2003). Molecular characterisation of fluoroquinolone resistant *Streptococcus pneumoniae* clinical isolates obtained from across Canada. Diagnostic Microbiology and Infectious Disease, 45, 63-67.

13. ACKNOWLEDGEMENTS

This opinion of the Scientific Committee on Veterinary Measures relating to Public Health is substantially based on the work of an *ad hoc* working group of the Committee. The working group included members of the Committee.

The working group was chaired by

- Prof. Johanna Fink-Gremmels

and included the following members:

- Prof. Jeanne Brugère-Picoux
- Dr. Hilde Kruse (rapporteur)
- Prof. Alexander M. Johnston