Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the use of antimicrobials for the control of *Salmonella* in poultry. [1]

(Question N° EFSA-Q-2004-079)

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SUMMARY

The existing Community legislation on food hygiene and control of zoonoses includes a number of provisions that seek to control and prevent the *Salmonella* contamination of foodstuffs. Targets for *Salmonella* spp. will be set progressively in different animal populations: breeding flocks of *Gallus gallus*, laying hens, broilers, turkeys and slaughter pigs. After each target is set, Member States will have to develop and submit national control programmes to the Commission for its approval. According to the Regulation, it may be decided to establish rules concerning the use of specific control methods in the context of the control programmes. The Regulation lays down that before proposing such rules on specific control methods, the Commission shall consult the European Food Safety Authority. The use of antimicrobials against *Salmonella* spp. is an example of such potential specific control methods.

Salmonella spp is widespread in poultry production in Europe. Prevalences vary considerably depending on country and type of production. Prevalences are lowest at the top of the production pyramid, i.e., the breeder stages. Poultry meat and eggs represent an important source of human infection with *Salmonella* spp. *S.* Enteritidis and *S.* Typhimurium are the most commonly reported serovars isolated from poultry, poultry meat products and human cases of salmonellosis.

Antimicrobial resistance in *Salmonella* spp. and other bacteria is an increasing public health problem. The risk to public health from the selection of resistant organisms depends on the likelihood of this event for a particular bacterium, the behaviour and prevalence of the bacteria, the antimicrobial in question, the type of resistance (transmissible or not, possibility of cross-resistance and co-selection), and type and stage of poultry production. The consequences of resistance to certain antimicrobials, especially fluoroquinolones and cephalosporins, are of particular concern, since these are critically important for therapy of human systemic bacterial infections

The basis for successful control of *Salmonella* infections in poultry farms are good farming and hygienic practices (including all the aspects covering feed, birds, management, cleaning and disinfection, control of rodents etc.) as well as testing and removal of positive flocks from production. In addition, antimicrobial treatment is regarded as an alternative measure to reduce the level of infection. The alternatives to antimicrobial usage in poultry are slaughter and heat treatment, depopulation or other potential treatments.

The advantages of antimicrobials used in poultry and listed below must be balanced against the risks associated with the development, selection and spread of antimicrobial resistance.

Any use of antimicrobials in poultry will increase the risk of emergence and spread of resistance in zoonotic bacteria such as *Salmonella* spp. and *Campylobacter* spp., as well as in animal pathogens and commensal bacteria. However, on the rare occasions when *Salmonella* causes clinical infections in poultry, antimicrobials may be useful in reducing morbidity and mortality. The use of antimicrobials is never totally effective

for the control of *Salmonella* spp. because it is not possible to eliminate all the organisms from an infected flock However, antimicrobial use may reduce the within-flock prevalence of *Salmonella* infection and the level of excretion, and reduce environmental contamination. Thereby the likelihood of spread to other flocks may be reduced and may limit the vertical transmission of *Salmonella* spp.

The use of an antimicrobial may select for resistance to other antimicrobials through cross-resistance or co-selection. There is also a danger that antimicrobial treatment may be used as a substitute for good hygiene and biosecurity and so perpetuate the persistence of *Salmonella* spp. infection in consecutive poultry flocks, which is less likely if infected flocks were slaughtered.

Valuable genetic material may be salvaged from infected breeding flocks through the use of antimicrobials to provide *Salmonella*- free eggs in order to establish a new *Salmonella*-free flock. In breeder flocks the risk of dissemination of residual *Salmonella* spp including resistant strains, through the production pyramid is high, compromising any potential advantage of treatment.

No specific advantages were identified in the case of laying hens. Some laying flocks may be persistently infected with *Salmonella* spp. so antimicrobial treatment presents a risk of maintaining a permanent infection cycle in the laying house as well as promoting the development, selection and dissemination of resistance.

If infected broiler flocks are not depopulated, antimicrobials may be useful as a short term measure for broiler chicks which have originated from an infected parent flock or contaminated hatchery to limit the extent of subsequent infection. Antimicrobial treatment of meat producing birds increases the risk of carcase contamination with resistant *Salmonella* spp, Campylobacter spp as well as resistant commensal bacteria, which may also transfer resistance genes to other bacteria;

Should antimicrobial resistant bacteria be already present, develop or be acquired, then the use of antimicrobials for the treatment of clinically infected flocks, for the prevention of *Salmonella* infection, or for the treatment of infected flocks without clinical signs, will enhance the selection and spread of resistant bacterial strains throughout the production pyramid.

For the prevention of *Salmonella* infection and for the treatment of infected breeding flocks in the absence of clinical signs, use of antimicrobials presents a risk of generation and wide dissemination of resistant organisms, through the breeding pyramid. Antimicrobial use in commercial flocks presents a risk of generation of resistant organisms which may contaminate eggs or meat and persist in the house to infect consecutive flocks of birds, whereas not using antimicrobials could lead to the introduction of *Salmonella* into the food chain.

Antimicrobial therapy can reduce the carriage and excretion of *Salmonella* spp. below the level of detection thereby reducing the diagnostic sensitivity of current monitoring programs, and so may interfere with the detection or confirmation of infection. The

misuse of antimicrobials may compromise the effectiveness of live bacterial vaccines, competitive exclusion cultures and probiotics.

The Panel concludes that from a food safety/public health viewpoint, using antimicrobials to control *Salmonella* spp. in poultry has little justification. Any use in exceptional circumstances on animal health and welfare grounds must recognize the consequences for public health.

The Scientific Panel on Biological Hazards recommends that the use of antimicrobials for *Salmonella* control in poultry should be discouraged due to public health risks associated with development, selection and spread of resistance. Their use should be subject to formally defined conditions that would ensure protection of public health. Such use must be fully justified in advance and recorded by the competent authority.

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BACKGROUND

Salmonella is one of the major causes of food borne illnesses in humans. According to the Commission's report on zoonoses[2] a total of 157 822 cases of human salmonellosis were reported by 14 Member States in 2001. Poultry meat and products thereof are regarded to be one of the major sources of these human food-borne infections.

Community legislation on food hygiene and control of zoonoses includes a number of provisions that seek to control and prevent the salmonella contamination of foodstuffs. These provisions cover the whole stable to table continuum. Measures to reduce salmonella prevalence in live animals is believed to be one of the most effective ways of reducing the contamination of foodstuffs and the number of human salmonellosis cases.

Council Directive 92/117/EEC[3] concerning protection measures against specified zoonoses and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxications is at the final stage of revision. It will soon be repealed and replaced by a Directive[4] on the monitoring of zoonoses and zoonotic agents and a Regulation on the control of salmonella and other specified zoonotic agents[5]. The proposed Regulation provides for the setting of pathogen reduction targets along the food chain, mainly for animal populations, and the establishment of national control programmes in order to meet these targets. *Salmonella spp*. is the primary target, in particular the serotypes considered to have public health significance. Targets will be set progressively in different animal populations: breeding flocks of *Gallus gallus*, laying hens, broilers, turkeys and slaughter pigs.

After each target is set, Member States will have to develop and submit to the Commission for its approval, national control programmes. According to the Regulation, it may be decided to establish rules concerning the use of specific control methods in the context of the control programmes. The Regulation lays down that before proposing such rules on specific controls methods, the Commission shall consult the European Food Safety Authority.

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2 European Commission : Trends and sources of zoonotic infections in animals, feedingstuffs, food and man in the European Union and Norway in 2001

3 O.J. L 62, 15.3.1993, p. 38; Directive as last amended by Directive 1999/72/EC of the European Parliament and of the Council (OJ L 210, 10.8.1999, p. 12)

4 O.J. L 325 Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agent, amending Council Decision 90/424/EEC and repealing Council directive 92/117/EEC.

5 O.J. L 325 Regulation (EC) No 2160/2003 on the control of salmonella and other specified food-borne zoonotic agents.

Treatment of the salmonella positive animals with antimicrobials is an example of such potential specific control methods. During the co-decision procedure on the Regulation, concerns have been expressed by the European Parliament in relation to the use of antibiotics in animal production and the related risk of development of resistance to antimicrobials. The Commission declared to the Parliament that it would ask for a scientific evaluation of the use of antibiotics as medicines, in the framework of salmonella control programmes, particularly in poultry.

As far as the current legislation is concerned, Directive 92/117/EEC laid down minimum monitoring and control measures against *Salmonella* in breeding flocks of *Gallus gallus* in its Annex III, section I. The original legislation required that flocks confirmed infected by *Salmonella* Enteritidis/ *Salmonella* Typhimurium (SE/ST) be eliminated. Following an amendment through Directive 97/22/EEC[6] of 22 April 1997, an option was introduced for Member States to waive the compulsory elimination under defined conditions. Certain restrictions have to be placed upon infected flocks, until it has been established to the satisfaction of the competent authority that the infection due to SE/ST is no longer present.

In the 7 Member States, whose salmonella control programmes in poultry have been approved by the Commission so far, this option is not used in breeding flocks.

On 26 June 2001 the Commission adopted a Community strategy against anti-microbial resistance (doc COM (2001) 333 final). This strategy comprises actions in all relevant sectors, including public health, veterinary and phytosanitary sectors.

The issue of antimicrobials was discussed in the following Community scientific reports and opinions:

Report of 11.11.1993 of the Scientific Veterinary Committee (SVC) on procedures for detecting salmonellae as zoonotic agents in general, on alternative methods for monitoring systems and, on possible methods for protecting poultry breeding flocks against salmonellosis (doc VI/3759/93-EN)

Report of 20.02.1995 of SVC on the measures required to control *Salmonella* in flocks of layers (doc VI/1726/95 rev2)

Opinion of 28.05.1999 of the Scientific Steering Committee on antimicrobial resistance http://europa.eu.int/comm/food/fs/sc/ssc/out50_en.html

Opinion of 26-27.03.2003 of SCVPH on the human health risk caused by the use of fluoroquinolones in animals. http://europa.eu.int/comm/food/fs/sc/scv/outcome_en.html

[6] OJ L 113, 30.4.1997, p. 9.

TERMS OF REFERENCE

The European Food Safety Authority is asked to

(1) Evaluate the advantages and disadvantages of the use of antimicrobials in the framework of programmes to control *Salmonella* in poultry, in particular taking into account the different types of flocks, such as *Gallus gallus* and turkey flocks as well as breeding, laying hen and broiler flocks.

(2) In view of the overall strategy against antimicrobial resistance, assess the risks that could result from the use of such antimicrobials for the
prevention of *Salmonella* infection in animals,
treatment of flocks infected with *Salmonella* without clinical signs,
treatment of clinically affected flocks

(3) Highlight any aspects related to the use of antimicrobials that may jeopardize a successful implementation of a programme to control *Salmonella*, in order for the Commission to take the best possible measures

SUPPORTING DOCUMENTS

Report of 11.11.1993 of the Scientific Veterinary Committee (SVC) on procedures for detecting salmonellae as zoonotic agents in general, on alternative methods for monitoring systems and, on possible methods for protecting poultry breeding flocks against salmonellosis (doc VI/3759/93-EN)

Report of 20.02.1995 of SVC on the measures required to control *Salmonella* in flocks of layers (doc VI/1726/95 rev2)

ASSESSMENT

The primary focus of this report is on safeguarding public health rather than intervention in animal health problems.

1. BACKGROUND INFORMATION

1.1. Epidemiology of non-typhoid salmonellosis in humans in Europe

Salmonella spp. are Gram-negative, facultative anaerobe, motile and rodshaped bacteria belonging to the family Enterobacteriaceae. At least 2,500 different serovars of *Salmonella* spp. are known and have been placed in two species; *S. enterica* and *S. bongori*. *S. enterica* is divided into six subspecies: *enterica, salamae, arizonae, diarizonae, houtenae* and *indica*. Names for *Salmonella* serovars (e.g., *S. enterica* subsp. *enterica* serovar Enteritidis is abbreviated to *Salmonella* Enteritidis) are only maintained for the subspecies *enterica* serovars, which account most of the *Salmonella* strains isolated from poultry and humans (see Brenner *et al*, 2000 for the *Salmonella* nomenclature).

S. Typhi and most *S.* Paratyphi (A, B and C) cause serious systemic infections in humans. Most of these serovars are specific human pathogens, and are transmitted directly or indirectly from humans to humans. Thus, animals are not a reservoir for these pathogens.

The zoonotic *Salmonella* spp. cause so-called non-typhoid salmonellosis that in humans usually presents as localized enterocolitis. The incubation period ranges from 5 hours to 7 days, but signs and symptoms usually begin 12 to 36 hours after ingestion of a contaminated food. The shorter incubation periods are usually associated with either higher doses of the pathogen or highly susceptible persons. Signs and symptoms include diarrhoea, nausea, abdominal pain, mild fever and chills. The diarrhoea varies from a few thin vegetable-soup-like stools to massive evacuations with accompanying dehydration. Vomiting, prostration, anorexia, headache, and malaise may also occur. The syndrome usually lasts for 2 to 7 days. Systemic infections sometimes occur, and usually involve the very young, the elderly or the immunocompromised. A fatal outcome is rare. The excreta of infected persons will contain large numbers of *Salmonella* spp. at the time of onset of illness. Those numbers decrease with the passing of time. Some patients become carriers, but some persons excrete non-typhi *Salmonella* spp. after three months. Non-typhoid salmonellosis can also result in sequelae, including reactive arthritis as well as neurological and neuromuscular illnesses.

The occurrence of antimicrobial resistance in *Salmonella* spp. has increased over the last decades representing a considerable public health concern. In developed countries it is well documented that antimicrobial resistance in *Salmonella* spp. in the food chain is associated with usage of antimicrobials in food animals (Mølbak *et al*., 2002). Thus, the use of antimicrobials in food animals exert a selective pressure promoting the development and spread of

antimicrobial resistance in *Salmonella* spp. that can be further transferred to humans through the food chain.

Human can acquire *Salmonella* spp. infections through the consumption of contaminated foods as well as contaminated drinking water. The SCVPH concluded that the food categories possibly posing the greatest hazard to public health include raw meat and some meat products intended to be eaten raw, raw or undercooked products of poultry meat, eggs and products containing raw eggs, unpasteurised milk and some products thereof. Sprouted seeds, unpasteurised fruit juices as well as home-made mayonnaise are also of major concern (SCVPH, 2003).

1.1.1. Serovars involved

Any serovar that is not animal host-adapted is considered capable of causing gastro-intestinal illness of varying severity in humans. The most frequently reported serovars involved in human salmonellosis in the EU are *S.* Typhimurium and *S.* Enteritidis, particularly phage type (PT) 4 (= PT 4) until 2002 (EC, 2002) and more recently, a range of other phage types including PTs 1 and 14b (O'Brien *et al*, 2004). *S.* Enteritidis and *S.* Typhimurium were also the most frequently reported serovars involved in outbreaks of salmonellosis in Europe in the period 1993-1998, being responsible for 77.1% of the outbreaks recorded and occurring in a ratio of approximatively 3:1 (FAO/WHO, 2001). The relative importance of serovars originating from poultry differs and dynamic changes are undergoing between regions and production type. *S.* Enteritidis predominantly originates from layers or egg products while *S.* Typhimurium originates from cattle, pigs and poultry in different proportions. The serovars responsible for human salmonellosis cases in European countries from 1993 to 2002 from various sources are presented in Table 1.

Table 1. Most frequently reported *Salmonella* serovars in humans based on laboratory surveillance data (WHO, 2001; EC, 2002, EC 2004).

Salmonella	**Year**							
serovar	**1993[1]**	**1994[1]**	**1995[1]**	**1996[1]**	**1997[1]**	**1998[1]**	**2000[2]**	**2002[3]**
S. Enteritidis	74%	77%	77%	79%	80%	84%	59%	67%
S. Typhimurium	20%	16%	17%	16%	15%	12%	13%	17%
S. Infantis	1.2%	1.1%	1.3%	0.9%	0.9%	0.6%	0.9%	0,7%
S. Hadar	0.4%	0.8%	0.8%	1.0%	1.0%	0.9%	1.8%	0.6%
S. Virchow	1.0%	1.1%	0.9%	0.6%	0.5%	0.4%	1.4%	0,5%
Other serovars	3.6%	4%	3%	2.5%	2.6%	2.1%	23%	14.2%

[1] WHO, 2001; [2] EC, 2002; [3] EC 2004.

1.1.2. Types of food involved

The contribution of the various food categories to the occurrence of domestically acquired human salmonellosis varies between countries depending on the prevalence of different *Salmonella* serovars in various food production chains, as well as consumption patterns and food preparation practices. Moreover, that picture will also change with time.

According to WHO (FAO/WHO, 2001), in Europe in the period 1993 – 1998, the incriminated food was identified in 1409 outbreaks caused by *S.* Enteritidis and in 188 outbreaks caused by *S.* Typhimurium. At least 76% of *S.* Enteritidis outbreaks reported were related to the consumption of "cooked" eggs, egg products or foods containing raw eggs such as ice creams or creams pastry fillings (Table 2). The role of eggs and products containing eggs in *S.* Enteritidis infections have also been established by several case-control studies (Table 3).

Several other foods have frequently been responsible for outbreaks caused by *S.* Typhimurium including meat and meat products (33%) – predominantly pork meat - and poultry meat products (10%) (Table 2).

Table 2. Types of food identified in the outbreaks caused by *S.* Enteritidis and by *S.* Typhimurium (WHO, 2001).

TYPE OF FOOD	PERCENTAGE CAUSED BY	
	***S.* Enteritidis**	***S.* Typhimurium**
Eggs and egg products	68	39
Cakes and ice cream	8	2
Meat and meat products	4	33
Mixed foods	4	2
Poultry and poultry products	3	10
Milk and milk products	3	2
Fish and shellfish	2	3
Other	8	9
Total (%)	100	100

The data on reported outbreaks or case-control studies alone are used to identify but not to quantify the contribution of the various sources to human salmonellosis. In Denmark, Hald *et al* (2004) developed a mathematical model

to calculate the number of domestic and sporadic cases caused by different *Salmonella* sero- and phagetypes as a function of the prevalence of these *Salmonella* types in the animal-food sources and the amount of food source consumed. The most important food sources were table eggs and domestically produced pork comprising 47.1% (95% CI: 43.3–50.8%) and 9% (95% CI: 7.8–10.4%) of the cases, respectively.

Table 3. Risk factors identified in case control studies on *S*. Enteritidis infections.

Year (Reference)	Country Cases and controls	Main risk factors
1988	United Kingdom	1. Consumption of raw shell eggs and products thereof
(Cowden *et al.*, 1989)	232 cases / 696 controls	2. Sandwiches containing mayonnaise
		3. Sandwiches containing eggs
		4. Lightly cooked eggs
1995	USA	Dining in restaurants that used significant more eggs than average
(Sobel *et al.*, 2000)	43 cases / 86 controls	
1996/1997	USA	1. Travelling outside the USA
(Kimura *et al*, 1998)	182 cases / 345 controls	2. Among non-travellers: eating runny eggs outside the home or eating chicken outside the home
1997/1999	Denmark	1. Foreign travel
(Mølbak and Neimann, 2002)		2. Among non-travellers: eating eggs or dishes containing raw or undercooked eggs
2003	United Kingdom [1)]	1. Consuming egg sandwiches outside the home
(O'Brien *et al*, 2004)	55 cases / 102 controls	2. Consuming sandwiches outside the home
		3. Eating eggs in Chinese restaurants
		4. Eating chicken dishes in Chinese restaurants

[1)] This concerns a so-called diffuse nation wide outbreak caused by *S*. Enteritidis phage type 14b. In a previous study it was shown that Spanish eggs were the most probable source.

1.2. General Structure of poultry production

The industrial production of poultry is very diverse. There are two main food production systems: poultry meat (carcasses and processed products), and eggs for consumption (table eggs) and further processing (egg products).

Various species are used in industrial poultry meat production: chickens (*Gallus gallus*), turkeys (*Meleagris gallopavo*), ducks (*Cairina moschata* and

Anas platyrhynchos) and guineafowl (*Numida meleagridis*), their importance varying with regions and food customs. Some alternative production systems also exist, such as organic and free-range production.

Production of poultry meat or eggs (Figure 1) is based on selection of male and female pure lineages on very precise genetic criteria, such as productivity, quality of products and resistance against disease. The selection methods assure a uniform quality of bird for further multiplication and production. Selection criteria differ according to the types of production. After the incubation time of eggs stemming from this first crossing, the chicks are raised in breeding steps, giving rise to chicks intended for fattening for poultry carcasses, and pullets for laying of eggs for human consumption. The selected offspring from these are then multiplied in great-grandparent flocks and grandparent flocks which are maintained at high health status. Chicks from grandparent flocks are used to populate parent flocks, e.g. broiler or layer breeder flocks, which are normally held by individual commercial companies. Eggs from these parent flocks are then hatched in commercial hatcheries to produce the commercial generation of birds.

Different genetic lines of birds are used for meat and egg producing flocks of chickens. Moreover, genetically male and female lines may be more specialised so as to contribute carcase characteristics and fecundity, respectively. There are also different genetic lines of birds for conventional and free-range or organic production systems.

The structure is "pyramidal". Every stage engenders a consequent reproduction of the number of individuals of the following stage (Figure 1): for example, at the selection step, every hen produces 30 to 50 chicks. Afterward, at the stage of breeding, this multiplication factor is increased and can reach 90 laying hens or 130 to 150 broilers. Because of this mode of production, theoretically every great-grandparent female (Elite) could be the origin of between 156,000 and 300,000 broilers or between 160,000 and 300,000 laying hens producing between 4.16×10^7 and 9.00×10^7 table eggs.

Intense genetic selection is carried out in primary breeding or elite flocks to achieve ongoing progress in terms of performance characteristics. These flocks are normally kept under conditions of extremely high biosecurity and in the case of chickens, normally in regions where there is a low prevalence of *Salmonella* spp. and low risk of other notifiable avian diseases that may threaten the long term survival of the flock.

Although these stages are physically separated in buildings and by the phase of hatching, this pyramidal structure can be the origin of an infectious agent, if transmission in the hatchery can occur.

There is a similar tiered structure for most turkey and duck production, but in many countries arrangements for more uncommon species and the organic production are less structured.

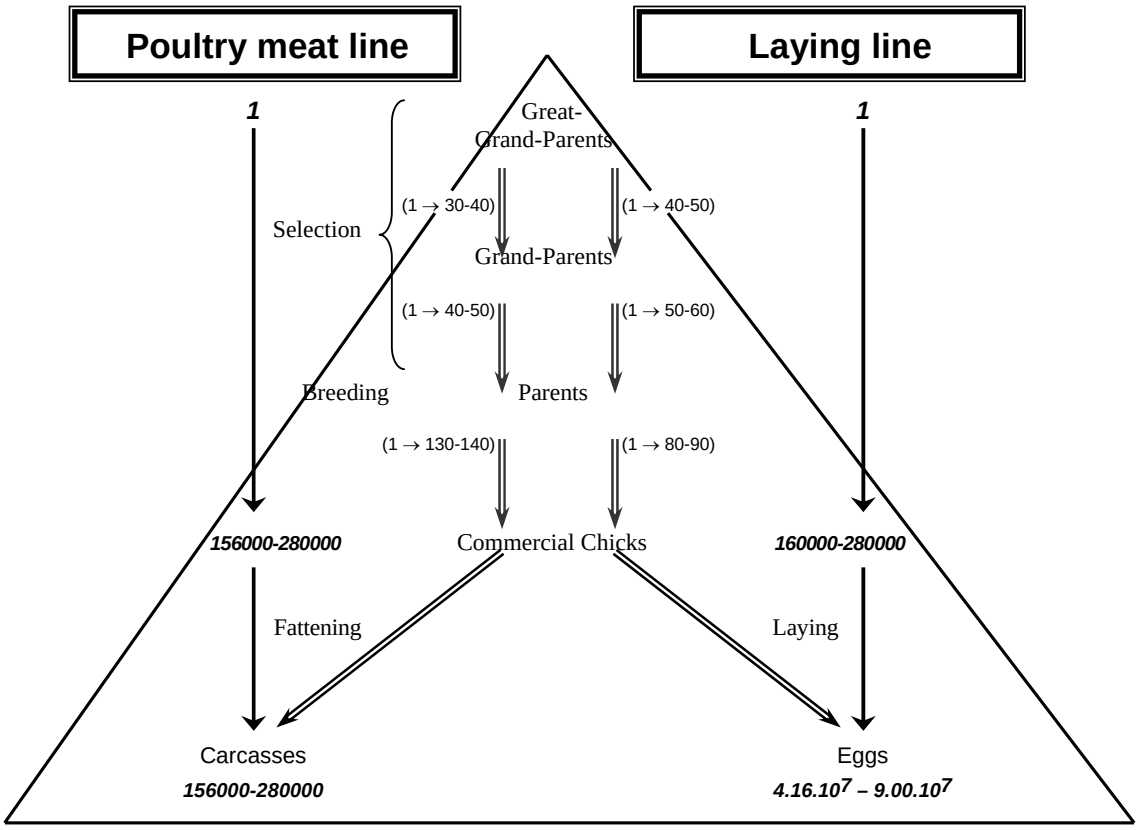


Figure 1. Simplified structure of poultry production

1.3. Occurrence of *Salmonella* spp. in poultry production

Salmonella spp. may contaminate many stages of food production, but the primary production of food animals remains the most important reservoir of *Salmonella* spp. entering the human food chain. The prevalence of *Salmonella* spp. in food animals may vary depending on the geographic region as well as on production systems and the stringency of control measures are introduced.

Due to the differences in monitoring schemes and methodologies employed, data from the various countries in the EU or other countries on the occurrence of *Salmonella* spp. in poultry production are difficult to compare. Consequently, interpretation of the data must take into account these differences. The prevalence of *Salmonella* in poultry presented in the sections below is taken from the reporting for the year 2002 according to the Directive 92/117/EEC from the various Member States as well as Norway.

1.3.1. Breeding flocks of Gallus gallus (chicken, hens)

Since 1998, the *Salmonella* control programmes in Denmark, Finland, Norway and Sweden have documented a low prevalence of *S.* Enteritidis and *S.* Typhimurium as well as other *Salmonella* serovars for breeding flocks of layers and broilers (< 1% prevalence of *Salmonella* spp.).

For the other EU countries, a decreasing trend in the prevalences of *S.* Enteritidis and *S.* Typhimurium for breeder flocks has been observed during the last years. In 2002, the reported flock prevalences for *S.* Enteritidis and *S.* Typhimurium ranged between 0% (Great Britain) and 6% (Greece) for broiler breeders. In 2002, the flock prevalences of *S.* Enteritidis were between 0% (Austria, France, Ireland and Great Britain) and 6.1% (Greece). In relation to distribution of serovars among the isolates from breeder flocks in 2002 (*Gallus gallus*), *S.* Enteritidis was the predominant serovar reported, representing 42% of all findings. For layer breeders, 63% of the isolates were *S.* Enteritidis, with *S.* Braenderup as the second most frequently reported serovar (14%). For broiler breeders, 42% of the isolates were *S.* Enteritidis, with *S.* Livingstone as the second most frequently reported serovar (7%). *S.* Typhimurium was reported in 4% of all isolates from breeding flocks (EC, 2004). This picture might be biased as in some countries only findings of *S.* Enteritidis and *S.* Typhimurium are notified in breeding flocks. As regards the serovars *S.* Infantis, *S.* Hadar and *S.* Virchow, which are among the top five serovars involved in human salmonellosis, only a few isolates were reported in poultry breeding flocks.

1.3.2. Laying hens and eggs for human consumption

Since 1996, the *Salmonella* control programmes in Finland, Norway and Sweden have documented that the prevalence of *Salmonella* spp. in laying flocks is below 1%. In these countries, the stringent control programme including a stamping out policy ensures that the egg production is virtually free from *Salmonella* spp.

In Denmark and Ireland, the control programmes document a decreasing prevalence of *Salmonella* positive flocks, mainly below 5% (EC, 2004).

In Austria, Germany, Spain, Greece, France, Italy, Great Britain, Northern Ireland, and the Netherlands, *S.* Enteritidis was the dominant serovar detected in 2002. In these countries, the prevalence of *Salmonella* positive layer flocks has varied between 1.5% and 37% during 2000-2002. In 2002, the reported flock prevalences for *S.* Enteritidis in laying hens ranged from 0.8 % (Germany) to 7.2 % (Spain), as opposed to 0.1% (Germany) to 0.7% (Greece) for *S.* Typhimurium (EC, 2004). In the countries where data on other serovars were available, the prevalence rate for “other serovars” ranged from 0% (the Netherlands) to 3% (Greece).

In 2000-2002, *Salmonella* spp. was detected in 0-10.4% of eggs, 0-7.6% of raw materials and 0-7.4% of egg products. For 2002, a *Salmonella* prevalence above 1% in table eggs was reported in four (Austria 1.1%, Greece 3.8%, Italy 3.1%, Spain 8.1%) out of eight reporting countries. In 2002, *S.* Enteritidis was the dominating serovar in egg and egg products positive for *Salmonella* spp. (73% of isolates), followed by *S.* Typhimurium (EC, 2004).

In England and Wales, in studies carried out between October 2002 and December 2003, *Salmonella* spp. was recovered from 4.1% of 1375 pooled samples (O'Brien *et al.,* 2004, in press). A recent survey in the UK, which sampled UK-produced eggs on sale in shops and markets, found that one in every 290 boxes of six eggs on sale had *Salmonella* contamination, compared with 1 in 100 in a 1995/96 survey (FSA, 2004). In Denmark, 0.07% out of 10,180 domestic shell eggs and 0.8% out of 4,900 imported eggs analysed were positive for *Salmonella* spp. in 2002 (Anonymous, 2004).

1.3.3. Broiler flocks and broiler meat

Although the Council Directive 92/117/EEC on zoonoses does not lay down requirements for monitoring in broiler flocks, several countries apply a monitoring scheme based on the sampling procedures from the above Council Directive. Since 1996, the *Salmonella* control programmes in Finland, Norway and Sweden have documented that the prevalence of *Salmonella* spp. in broiler flocks generally is below 1%. In Denmark, the monitoring has shown a decreasing prevalence of *Salmonella* positive broiler flocks with 1.5% in 2002 (0.2% *S.* Typhimurium). The situation in the broiler flocks is reflected in the *Salmonella* situation in poultry meat. The prevalence at slaughter was 0% for Norway, 0.07% for Sweden and 5.5% for Denmark, the prevalence at processing 0.2% for Finland. In Austria, Germany, Spain, Greece, Italy and the Netherlands, the prevalence of *Salmonella* positive broiler flocks ranged from 1.2% to 22.8% in 2000-2002 (EC, 2004).

Regarding serovar distribution in broilers in 2002, *S.* Paratyphi B var. Java was predominant (20% of isolates), attributable to the situation in the Netherlands in 2002, followed by *S.* Enteritidis (11%). Each of the serovars *S.* Infantis, *S.* Virchow, S. Livingstone, *S.* Mbandaka, *S.* Typhimurium, *S.* Senftenberg and *S.* Hadar had a share between 3-6%.

In 2000-2002, *Salmonella* spp. was detected in 0-34% of samples (Greece 2002, 34%). Regarding serovar distribution among *Salmonella* spp. isolates from poultry meat, *S.* Enteritidis and *S.* Typhimurium were predominant (11% of isolates each) followed by *S.* Kentucky (7%), *S.* Paratyphi B var. Java (6%) and *S.* Livingstone (3%) in 2002 (EC, 2004).

1.3.4. Other poultry (excluding Gallus gallus)

Breeding flocks

In turkey breeding flocks, in 2002, no *S.* Enteritidis or *S.* Typhimurium were detected in the monitoring programme in Finland, Sweden, Norway, the Netherlands, and Ireland. In France, 1 % of the flocks were *Salmonella* positive during the production period. In Germany and Italy, no positive turkey breeding flocks were reported within the voluntary investigations. In Sweden, Norway and France, where geese breeders are covered by the monitoring programme, no flocks were positive in 2002. In France, 36% of the duck breeding flocks were infected with *Salmonella* spp., which is an increase compared with 14.8% in 2001 (EC, 2004).

Production

In 2002, *Salmonella* spp. was not detected in turkey flocks in Sweden and Norway, whereas the flock prevalence was 0.5% in Finland (0.2% *S.* Enteritidis), 8.6% in Ireland (no *S.* Enteritidis or *S.* Typhimurium), and 8.4% in Denmark (1.6% *S.* Typhimurium). In turkey meat collected at retail in Denmark in 2002, no *Salmonella* spp. were detected. In Germany, in the voluntary sampling, 9.6% of flocks and 10% of samples of turkey meat were *Salmonella* positive. In Austria, 5.9% of the samples tested were positive for *Salmonella* spp. In a study, run in the Veneto Region of Italy, 61% of the flocks were positive for *Salmonella* spp.

In 2002, no *Salmonella* spp. was detected from geese flocks in Norway, whereas 2.9% of flocks in Sweden were positive (*S.* Enteritidis). In Austria, 6.8% of the geese flocks tested were *Salmonella* positive and in Germany 8.7%. In Norway and Sweden, no *Salmonella* positive commercial duck flocks were identified. In Denmark, *Salmonella* spp. was isolated in a high proportion of duck flocks tested (55%). In several incidents, more than one serovar was isolated. *S.* Anatum continued to be the most frequently isolated serovar. In Austria and Germany, 16.7% and 10.6%, respectively, of the duck samples tested were *Salmonella* positive. In Great Britain, 235 incidents were reported from ducks. *S.* Indiana (26.4%) was the most common serovar, followed by *S.* Orion (13.2%), *S.* Binza (12.7%) and *S.* Hadar (11.5%). In Northern Ireland, *S.* Mbandaka and *S.* Budapest were isolated from ducks.

Other poultry species, such as guinea fowl, ostriches, partridges, quails, and pheasants were tested for *Salmonella* spp. in some countries in 2002. Results show that all types of poultry can be infected with *Salmonella* spp. and that both *S.* Enteritidis and *S.* Typhimurium may be present.

1.4. Clinical *Salmonella* infections in poultry

Salmonella enterica subsp.*enterica* can be divided in two broad groups of serovars on the basis of pathogenesis and infection biology. One group consists of a small number of serovars that cause severe systemic typhoid-like disease in a restricted range of hosts. In poultry this group essentially consists of the serovars Pullorum and Gallinarum and the clinical diseases caused by these serovars are called pullorum disease and fowl typhoid. The other group comprises a large number of serovars that colonize the alimentary tract or cause

gastrointestinal disease in a wide range of hosts and are called paratyphoid infections.

The *Salmonella* serovar Gallinarum (which now includes Pullorum) causes outbreaks of disease in poultry with high morbidity and mortality. The clinical signs of pullorum disease and fowl typhoid are well known and have been reviewed recently (Shivaprasad, 2003). These diseases are rare in commercial poultry in the EU and will not be considered further in this report.

Paratyphoid infections in contrast are common in poultry in the EU. As opposed to Pullorum/Gallinarum, these paratyphoid infections are mostly subclinical in poultry. Nevertheless under certain conditions, some non-typhoid infections may cause severe clinical disease and mortality (Gast, 2003). Information in the literature on the serovars and the conditions leading to clinical paratyphoid is scarce. The outcome of these infections appears to depend not only on the serovar and the strain infecting the birds, but also on the infection dose, the presence of concurrent disease and the host (age and breed).

In day-old chicks non-typhoid infections can lead to severe morbidity and high mortality, while older birds may experience intestinal colonization and even systemic dissemination without significant morbidity and mortality (Gast and Beard, 1989; Desmidt *et al*., 1997). In adult laying hens, only occasional mortality and mild clinical signs including slight depression and mild diarrhoea lasting for only three days has been reported after experimental infection with *Salmonella* Enteritidis (Kinde *et al*., 2000). Adult birds in turn become highly susceptible to the infection again when moulted (Corrier *et al*., 1997). During moulting, *Salmonella* Enteritidis infection may lead to intestinal inflammation (Holt, 2003). This age related difference in susceptibility to non-typhoid is observed with many different serovars. Experimental infection of day-old chicks and 4 weeks old chickens with *Salmonella* Hadar, however, lead to similar excretion patterns (Desmidt *et al*., 1998a).

Morbidity in clinical non-typhoid infection is characterized by one or more of the following clinical signs: anorexia, adipsia, huddling together, ruffled feathers, reluctance to move, somnolence, dehydration, white scours and pasted vents (Marthedal, 1977). In the chronic stage retarded growth of some birds is usually the only obvious sequel (Desmidt *et al*., 1998a). Many non-typhoid serovars do not seem to cause any clinical signs under any condition. They temporarily colonize the gut and disappear within days or weeks (Heyndrickx *et al*., 2002). Some serovars however may colonize internal organs for weeks (Van Immerseel *et al*., 2004).

The above mentioned clinical signs and mortality have been reported only for a limited number of serovars, including among others: Enteritidis (Desmidt *et al*., 1997), Typhimurium (Barrow *et al*, 1987a; Bumstead and Barrow, 1988), Hadar (Desmidt *et al*., 1998a), Heidelberg (Roy *et al*., 2001). The occurrence and severity of clinical signs is not only serovar dependent, but also strain dependent. Experimental infection of newly hatched specific pathogen free

chicks with certain strains of Salmonella Typhimurium may lead to 100% mortality (Barrow *et al*., 1987a), while other strains of the same serovar induce much lower mortality rates. Experimental infection of newly hatched chicks with different strains of Salmonella Enteritidis may also cause different mortality rates (Dhillon *et al*., 1999). Differences in natural resistance against *Salmonella* infection between different lines of chickens have been reported (Bumstead and Barrow, 1988 and 1993). Certain lines are more resistant than others to intestinal carriage (Duchet-Suchaux *et al*., 1997) or to systemic infection and mortality (Bumstead and Barrow, 1988). An inverse correlation has been reported between severity of caecal infection and severity of systemic infection in different broiler lines (Kramer *et al*., 2001).

In adult laying hens, the important serovar is Enteritidis. Some *Salmonella* Enteritidis isolates cause a decrease in egg production after experimental oral infection (Gast, 1994). Most isolates however do not. In naturally infected laying flocks also, the egg production remains within the normal range (Awad-Masalmeh and Thiemann, 1993). Until the present day it is still unclear how serovar Enteritidis preferentially can infect hens' eggs without causing any clinical signs and without a drop in egg production. It is not until the complete pathogenesis of egg infection will be unravelled that truly efficient and targeted measures can be taken to prevent egg contamination (De Buck *et al*., 2004).

1.5. Detection methods of *Salmonella* spp. in poultry

Salmonella monitoring in poultry is based on periodic testing of flocks by means of different methods, with the aim of detecting positive flocks, assessing the prevalence of infected flocks or detecting changes in prevalence. The most frequently used methods are bacteriological and serological ones.

1.5.1. Bacteriological testing

These methods provide information on the current status of birds i.e. if they are excreting *Salmonella* spp. at the level that is possible to be detected by the sampling and the analytical method used. However, these methods are most suitable for the diagnosis of recently infected flocks when faecal excretion is high, while their diagnostic sensitivity may be too low to detect infected flocks later in the course of infection when only few birds excrete intermittently. In particular, excretion of *Salmonella* may be reduced in vaccinated flocks (Davies and Breslin, 2004).

Bacteriological testing can be performed on animal samples (faeces, cloacal swabs, organs, eggs) or on environmental samples. In the first case different sampling schemes can be used, depending on the aim of the monitoring. A sampling scheme aimed at assessing the prevalence of infected flocks in a country or area, must take into account:

- the expected prevalence of infected flocks;

- the expected prevalence of positive (or shedding) birds within the flock;
- the desired level of accuracy and confidence limits.

By environmental monitoring it is possible to assess the prevalence of contaminated flocks with greater sensitivity. Sampling schemes must take into account the expected prevalence of contaminated flocks and the desired level of accuracy and confidence limits.

In general, in the case of animal testing, the lower the within flock prevalence, the higher the number of samples to be taken. In practice sampling schemes are not designed in order to assess the prevalence, but to find at least one positive sample if the prevalence is above a certain level. Generally, 60 single samples are taken, in order to detect a within flock prevalence of 5% or more. If faecal samples or cloacal swabs are taken and cultured individually, the within flock prevalence corresponds to the percentage of animals shedding detectable levels of *Salmonella* at the moment of sampling.

According to the Commission Decision 2003/644/EC7, "the microbiological testing of the samples for *Salmonella* spp. should be carried out to the standard of the International Organisation for Standardisation (ISO 6579: 1993) or revised editions, or by the method described by the Nordic Committee on Food Analysis (NMKL method No. 71, 1991) or revised editions."

The ISO 6579 and the NMKL 71 procedures comprise several culture steps (pre-enrichment, selective enrichment, plating out, confirmation). Both procedures are intended for the detection of *Salmonella* spp. in food and feeding stuffs. For other type of samples, like faeces or environmental samples these procedures may be less suitable. For faecal samples it has been shown that replacement of one or both of the selective enrichment broths of the mentioned procedures by a semi-solid agar medium would lead to a higher detection rate of *Salmonella*.

The Sub Committee 9 (SC9: Microbiology) of ISO Technical Committee 34 (TC34: Food products) held in April 2004, agreed to prepare an annex to ISO 6579 for the detection of *Salmonella* from animal faeces and other samples such as dust in the primary production stage. In this annex the use of Modified Semi-solid Rappaport Vassiliadis agar (MSRV) as the only selective enrichment medium will be prescribed. This will facilitate sensitive monitoring at reduced cost compared with the full ISO procedure.

Beside the 'traditional' culture methods some countries also use other alternative methods. Some countries use PCR techniques, either as the detection

[7] Commission Decision of 8 September 2003, establishing additional guarantees regarding *Salmonella* for consignments to Finland and Sweden of breeding poultry and day-old chicks for introduction into flocks of breeding poultry or flocks of productive poultry.

method after a non-selective enrichment of the sample, or as confirmation method after selective enrichment (on e.g. semi-solid agars).

Enzyme immunoassay based (screening) techniques are also used for the detection of *Salmonella* antigens. Several systems are commercially available and the tests may be performed automatically.

1.5.2. Serological testing

During infection of poultry with *Salmonella*, the immune system will respond to the infection by antibody production towards antigenic determinants or by activation of a cellular immune response, or both. The production of antibodies during the course of an infection is usually referred to as a "serological response", meaning that antibodies may be detected in serum from blood samples of infected animals.

Serological monitoring is based on the same statistical criteria used for bacteriological monitoring, with the difference that the prevalence of reactors is assessed, instead of the prevalence of animals shedding *Salmonella* spp. Serological methods may be used in combination with bacteriological testing in order to increase the sensitivity of results. Due to infection dynamics bacteria may not always be easy to recover from infected flocks while an antibody response may persist for several months even though bacterial excretion is low. On the other hand, at the onset of infection antibodies may not yet have evolved and thus recently infected flocks may escape detection by serology alone. It must also be considered that the use of vaccines can lead to positive serological reactions, unless suitable discriminatory tests are applied.

The pandemic of *Salmonella* infections spreading in poultry flocks generated a worldwide need for research and development of detection methods, and almost all poultry-producing countries have looked into the use of serological methods for this purpose. The area has been intensively investigated for *S.* Enteritidis and to a lesser extent for *S.* Typhimurium, while research has been sporadic for other serovars infecting poultry.

Principally, the antigenic determinants of *Salmonella* spp. employed for this development are of two kinds: the surface structure of the bacterial cell wall contains lipopolysaccharides (LPS), and the flagellae contain protein structures, both of which are able to stimulate a production of antibodies during infection.

In the late 1980's and in the 1990's many serological tests for *Salmonella* spp.in poultry based on LPS-determinants in an ELISA format were published (Nicholas and Cullen, 1991; Van Zijderveld *et al*., 1992). Later, ELISAs based on flagellum proteins have been developed against *Salmonella* Enteritidis and *Salmonella* Typhimurium (Feberwee *et al*., 2001).

Several tests based on LPS or g,m-flagellin are commercially available. These are not entirely specific for *S.Enteritidis or S.Typhimurium* as they may detect also other serovars exhibiting similar LPS or g,m-flagellin antigens.

Serological ELISA tests have been developed as in-house methods in a number of countries and are also in use in national *Salmonella* control programmes, for example a mixed LPS ELISA is used for monitoring egg yolk antibodies in Danish laying flocks. However, as these have not been validated and approved by international validation bodies they are not available as international standards, in contrast to bacteriological detection methods for *Salmonella*

1.6. Controlling *Salmonella* spp. in primary production

Good Farming and Good Hygienic Practices (GFP and GHP) are examples of measures that can be applied in the control of *Salmonella* spp. However, in some occasions, vaccination and the use of antimicrobials are possible measures to control the presence of *Salmonella* spp. in poultry flocks. http://www.efsa.eu.int/science/biohaz/biohaz_opinions/721_en.html

1.6.1. Biosecurity

Biosecurity is defined as a health plan or measures designed to protect a population from transmissible infectious agents (Anonymous, 1999). This embodies all measures which can or should be taken to prevent viruses, bacteria, fungi, protozoa, parasites, disease carriers (rodents, insects, wild birds, people, equipment, etc) from entering and endangering the health status of a population.

In the poultry industry, biosecurity measures are used, for example, to minimise the risk of *Salmonella* spp. entering poultry farms and associated enterprises such as feed mills and hatcheries. Comprehensive biosecurity measures are costly in terms of capital equipment, use of disinfectants and other antibacterials, testing and labour. Measures include e.g. dedicated boots (and, in some cases, protective oversuits) for each house, facilities and protocols for hand hygiene, step-over barriers between a 'clean' and 'dirty' part of the house service area or ante-room and improved tidiness outside the house, including in-filling of areas where water can pool and improved drainage.

Maximum level of biosecurity is only possible where there is a high value product and where the consequences of *Salmonella* spp. being transmitted to customers are severe. Such measures are normally only applied in full in primary breeding and grandparent flocks and include heat treatment of feed at higher temperatures. Feed is also often tested for *Salmonella* spp. using rapid methods before delivery to farms. Feed mills are monitored by process and environmental monitoring as well as testing ingredients and finished products. There is extremely frequent and comprehensive monitoring for *Salmonella* spp. on farms and in hatcheries.

Ideally staff infected with *Salmonella* spp. should not come in contact with birds while they are excreting *Salmonella* spp. Visitors may also be asked to provide a negative faecal test result before being allowed on to the premises. Entry to the premises is via a hygiene barrier where showering in and out and use of disposable or site-dedicated protective clothing is required. Equipment used by contractors is either supplied by the company or fumigated on entry to the farm. Other farm inputs such as litter are also carefully sourced to minimise risk, tested and usually treated with antibacterial substances such as organic acids or formaldehyde/acid combinations.

The all-in/all-out production on a whole farm basis is one of the basic principles of effective biosecurity; it is applied in the commercial sector, but is often not possible on primary breeding farms because of the need to maintain and evaluate small groups of birds of high genetic potential. Such strict biosecurity applies in broiler primary and grandparent breedings in most European countries but measures may be less strict in grandparent flocks of some layer breeders, turkeys and ducks (Davies *et al.*, 1998; Davies *et al.*, 2003) where there may be farms or hatcheries which are not completely dedicated to grandparent production (e.g.: eggs from parent flocks may be hatched in the same premises as eggs from grandparent flocks). At the parent level, in conventional but not organic production, all-in/all-out production is normal.

Many of the biosecurity principles described above are applied, but at a lower intensity because of cost. However it is necessary that strict all-in/all-out production is applied so the necessary actions can be applied to ensure that *Salmonella* spp. does not persist for more than one flock cycle since it is possible to totally depopulate farms, remove all contaminated material, wash, disinfect and test to ensure that decontamination has been successful before restocking houses. In practice there has sometimes been insufficient time to complete this effectively before restocking. In particular, carriage of *S.* Enteritidis and to a lesser extent, *S.* Typhimurium and other serovars in breeding, mice populations harboured in dropping pits, storage areas and wall and roof insulation within the house has resulted in a high level of persistent infection.

In commercial broiler production improvements in the *Salmonella* spp. status of breeding flocks, feed control and improved cleaning and disinfection procedures can reduce *Salmonella* spp. to low flock and individual prevalence. At this time there is considerable interest in further improving on-farm biosecurity to reduce the prevalence of *Campylobacter* spp. and the introduction of viral diseases such as avian influenza.

Biosecurity in large-scale turkey production is of a similar standard but there are considerable problems with application of these measures on commercial duck farms and commercial laying farms (especially in multi-age in cage laying flocks). On cage layer farms movement of mice and other rodents, flies, egg belts and personnel can spread *S.* Enteritidis between houses despite

vaccination (Davies and Breslin, 2003a). Mice and poor cleaning and disinfection are also responsible for persistence of infection on the farm (Davies and Breslin, 2003b). All biosecurity programmes should be supplemented by genuinely effective monitoring to confirm their effectiveness.

1.6.2. Feed and Water Treatments

The basis of production of feed which is minimally contaminated with *Salmonella* is GMP and HACCP from harvest to delivery (Cooke, 2002). It is however not possible to totally exclude all sources of contamination so heat treatment is commonly used to decontaminate the final product. A temperature of 85°C for 2 minutes has been recommended for reliable de-contamination but, in practice, shorter conditioning times may be used. The increasing use of expansion and extrusion systems operated at high temperatures and often followed by a further pelleting stage ensures sufficient heat treatment for all but the most exceptionally highly contaminated ingredients. There is however a problem in some feedmills which is caused by recontamination in pellet or meal coolers which may persist for years or may be a more transient contamination caused by environmental dust from ingredient processing (Davies and Hinton 2000; Jones and Richardson 2004). Feeds for commercial layers are normally not pelleted or heat treated in many countries and whole grain may be fed to broilers without heat treatment. In some cases organic acids or formaldehyde treatment is used to minimise the risk of contamination and irradiation could theoretically be used but in practice this is restricted to treatment of special rations for laboratory animals.

A wide range of feed and water additives for the control of *Salmonella* spp. in poultry are described but most require more large scale field evaluations (van Immerseel *et al.*, 2002). In feed, preparations of organic acids can reduce the chance of flock infection both from contaminated feed and environmental challenge (Humphrey and Lanning, 1988; de Olivera *et al.*, 2000) but the efficiency of different products varies (Hume *et al.*, 1993) and those containing the highest levels of free-formic acid in a liquid application appear to perform best.

Treatment of water supplies with oxidising acidic agents, such as hydrogen peroxide/peracetic acid or lactic acid (Byrd *et al.*, 2001) or sodium chlorate and sodium nitrate (Jung *et al*, 2003) appears to have a beneficial effect on broiler contamination at slaughter and could be investigated in a wider ranger of situations.

1.6.3. Competitive Exclusion

Under free-range or non-intensive production systems, newly hatched birds acquire a variety of intestinal bacteria during their first few days of life from their local environment. Colonization of the intestine by such innocuous bacteria prevents the intrusion of *Salmonella* and other undesirable bacteria.

Such supression by the normal flora is known as "competitive exclusion" (Nurmi and Rantala, 1973; Pivnick and Nurmi 1982; Schneitz and Mead 2000).

In some countries application of competitive exclusion products, which are undefined or partially defined cultures derived from poultry intestinal microbiota (Nurmi and Rantala, 1973), have been widely used as part of general *Salmonella* control programmes (Wierup *et al* 1988, Wierup *et al* 1992). Currently there are difficulties with the use of undefined competitive exclusion cultures in some member states because of difficulties in the authorisation procedures (feed additives *versus* veterinary medicinal products).

A variety of different commercial products are available and these appear to have different levels of efficacy (Nakamura *et al.*, 2002; Ferreira *et al.*, 2003). The effectiveness is also related to the level of challenge but even when this is high there is still often usually some reduction in the prevalence of infection in individual birds and the numbers of *Salmonella* organisms excreted. This effect can be used to sequentially reduce the level of excretion and environmental challenge in consecutive flocks to the point when total elimination is more likely (Mead, 2000). Wider studies are needed to fully define this and further developments are in progress (Andreatti *et al.*, 2003). To be maximally effective competitive exclusion should be administered shortly before a potential exposure to *Salmonella* spp., so administration by spray at the hatchery is generally superior to water administration on farm (Mead, 2000; Patterson and Burkholder, 2003).

1.6.4. Probiotics and Prebiotics

Probiotics are claimed to have beneficial effects on the healthy individual (better performance) as well as positive effects on the prevention of intestinal disorders and the microecology of the gut (Fuller, 1989). They are applied as feed additives in animal husbandry. *Salmonella* spp. are a main target of the preventive effect. Probioitic strains applied belong mainly to the genera *Lactobacillus* or *Enterococcus* as well as to *Bacillus* or *Saccharomyces*. Their clinical relevance has been tested in several studies in humans (Marteau and Rambaud, 1993; Saxelin, 1997). Clinical effects as well as growth performance have been studied in farm animals including poultry, especially for *E. faecium* strains (Gutzwiller and Wyss, 1988; Bue *et al.*, 1990). A second field of application is to support the therapy of clinically affected animals (Charteris *et al.*, 1997), especially prevention of superinfections after antibiotic therapy (e.g. against *Salmonella* spp.), therapy of diarrhoea (bacterial or other) etc. This application can help to avoid therapy with antimicrobials.

The application as feed additives is strictly regulated within the EU. Concerning safety aspects no relevant antimicrobial resistances should be harboured by the probiotics and they should not be able to transfer resistant genes.

There has been no systematic investigation of the effect of probiotics on the control of *Salmonella* spp. in poultry.

Prebiotics, ie. nutrients designed to influence the intestinal flora in a positive way may also be used but there is limited information on their effect on *Salmonella* colonisation in the field. In experimental "in vivo" trials, protective effects of fructooligosaccharides have been shown with respect to *Salmonella* colonization of the chicken intestine (Bailey *et al*, 1991).

1.7. EC approved *Salmonella* control programmes

Council Directive 92/117/EEC on zoonoses provides for control schemes for *Salmonella* in breeding flocks of *Gallus gallus*, which are to be implemented by all Member States. By the end of 2003, the Commission had approved the national *Salmonella* control programme of seven Member States (Austria, Denmark, Finland, France, Ireland, Sweden and the Netherlands). In addition, the EFTA Surveillance Authority has approved the Norwegian plan. These programmes vary in particular in relation to the types of animal populations and *Salmonella* serovars covered. All approved control plans cover at least the breeding flocks of *Gallus gallus* in addition to some other poultry flocks (i.e., breeding flocks of another poultry species, flocks of laying hens or broilers), or another animal species. While Austria, Denmark, Finland, Sweden, the Netherlands and Norway target all *Salmonella* serovars, other countries restrict their control programme to *S.* Enteritidis and *S.* Typhimurium. Additionally, in some Member States, salmonellosis or all *Salmonella* isolations in animals are notifiable.

The Nordic countries where the first to demonstrate that application of control programmes can reduce the prevalence of *Salmonella* in poultry (e.g. Wierup *et al* 1988; Wegener *et al*, 2003;. Maijala *et al* (in press).

2. OCCURRENCE OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA* SPP. IN POULTRY PRODUCTION IN THE EU

Antimicrobial-resistant *Salmonella* spp. in animal production have been reported since the 1960s (Anonimous, 1969). The occurrence of resistance seems to have increased over the years, and is likely to be associated with the selective pressure exerted by the use of antimicrobials (Cohen, 1992) There are large variations between regions, sectors, and sources and the acquisition of resistance seems to vary between different serovars (EC, 2003a).

Antimicrobial-resistant *Salmonella* spp. are commonly isolated from different types of food animals and food products throughout Europe (EC, 2003). Over the last decade, clones of *Salmonella* spp. with multiple drug resistance have been distributed widely in many European countries; in particular multi-resistant *S.* Typhimurium definitive phage types (DTs) 204b and 104.

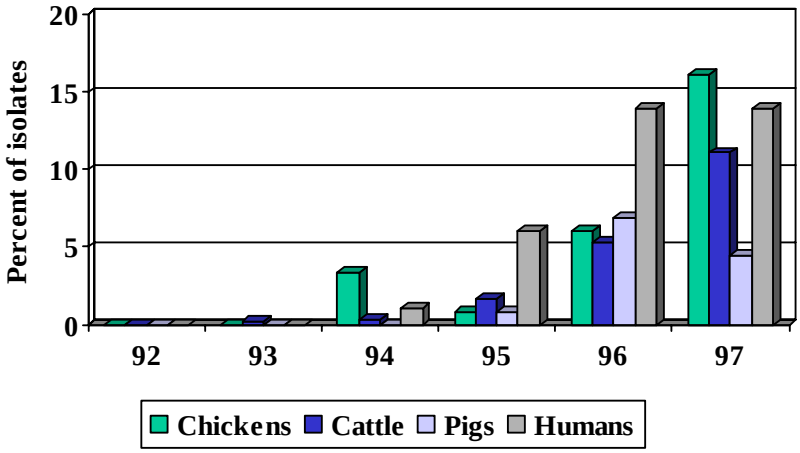
For 2002, 15 countries provided information on the occurrence of antimicrobial resistance in *Salmonella* spp. from food animals to the EC (EC, 2004). Quite

large country differences in the resistance prevalences and patterns were observed. Due to differences in sampling strategies, methodologies applied, and breakpoints used, comparisons needs to be done with great caution.

In general, resistance to tetracycline seems to be common in *Salmonella* strains from food animals in the European Union in 2002. Also, resistance to streptomycin, sulphonamides and ampicillin were often observed. Although fluoroquinolone resistance in many countries remains infrequent, resistance to nalidixic acid, which is an indicator of developing resistance to fluoroquinolones, was observed by most reporting countries.

As regards *Salmonella* isolates from poultry in 2002, resistance to tetracyclines, ampicillin, nalidixic acid, sulphonamides and streptomycin dominated. Five out of nine reporting countries observed resistance to fluoroquinolones (Finland, Greece, Italy, Portugal, and Spain).

Figure 2 Quinolone-resistant *Salmonella* Typhimurium DT 104 (UK, 1992 – 97) (Fluoroquinolones were licensed for use in food production animals in the UK in Nov 1993).



Resistance to different types of antimicrobials, including quinolones, has become quite common among *S.* Typhimurium and many strains are multi-resistant (EC, 2003; EC, 2004). In several European countries as well as North-America, a multi-resistant clone of *S.* Typhimurium DT 104 (MR-DT 104) became epidemic during the 1990s. MR-DT 104 has been isolated from many different food animals including cattle, pigs, sheep, and poultry. Infection in humans is generally foodborne. MR-DT 104 typically exhibits resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines (ACSSuT). Since the mid-1990s, the occurrence of resistance to quinolones has increased in MR-DT104 isolates. In UK, the emergence of quinolone-resistant MR-DT104 in poultry, cattle, pigs and humans (figure 2) followed soon after the licensing of enrofloxacin for use in food-production animals, in November

1993 (EMEA, 1999). In 2001 in the UK, overall 19.8% of DTs104 and 104b isolates were resistant to nalidixic acid compared to 11.7% in 2000, an increase of approximately 70% (EC, 2003) but there has subsequently been a reduction in the level of resistance.

In contrast to *S.* Typhimurium, *S.* Enteritidis isolates are, in general, susceptible to most antimicrobials, but Spain and Portugal reported relatively high prevalences of resistance to tetracycline, ampicillin, and chloramphenicol/florfenicol in 2002. It should be noted that resistance to quinolones in *S.* Enteritidis from cases of human infection is emerging in many EU countries (Mølbak *et al.*, 2002; Threlfall *et al.*, 2003 a, b), and also in poultry (EC, 2002; EC, 2004). In 2002, detection of nalidixic acid-resistant isolates from poultry was reported from Austria (5%), France (3%), Greece (41%), Denmark (23%), and Portugal (61%). In Denmark, resistance to nalidixic acid in *S.* Enteritidis increased from 0% in 2001 to 23% in 2002. Usage of fluoroquinolones in food animals in Denmark decreased markedly in 2002 and the increase in resistance was most likely as a result of clonal spread caused by trade in day-old chicks carrying nalidixic acid-resistant *S.* Enteritidis. This illustrates how the association between usage of antimicrobials and occurrence of resistance may be confounded by other factors, such as transmission of resistant bacterial strains between premises. In 2002, quinolone resistance was detected in 1% of poultry *S.* Enteritidis isolates from Italy, 5% from Spain and 13% from Portugal.

For serovars other than *S.* Enteritidis and Typhimurium, the reported resistance prevalences for poultry *Salmonella* spp. varies between serovars and countries. In 2002 in EU, resistance to tetracycline, ampicillin, and streptomycin was observed in *S.* Heidelberg, *S.* Indiana, *S.* Infantis, *S.* Kottbus, and *S.* Senftenberg. The prevalence of nalidixic acid resistance was 0% for *S.* Indiana from France, 28% for *S.* Heidelberg isolates in Austria, 26% for *S.* Infantis in Austria, 82% for *S.* Kottbus in France, 9% for *S.* Senftenberg in Austria, and 27% for *S.* Senftenberg in France. For *S.* Senftenberg, France reported 2% fluoroquinolone resistance, 13% chloramphenicol resistance, 24% tetracycline resistance, and 28% streptomycin resistance, whereas Austria reported 0% for these agents (EC, 2004).

In the Netherlands, *Salmonella* Paratyphi B variant (var.) Java increased in poultry from less than 2% of all isolates before 1996 to 60% in 2002. 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%. In the Netherlands, *S.* Paratyphi B var Java from poultry has been reported as becoming less susceptible to ciprofloxacin (van Pelt *et al.*, 2003).

In a study of isolates of *Salmonella* spp from human cases of salmonellosis in 10 European countries in 2000, 14% of 23 000 isolates exhibited resistance to quinolone antimicrobials, with 13% of S. Enteritidis, 8% of S. Typhimurium,

57 % of S. Virchow and 53% of S. Hadar exhibiting such resistance (Threlfall *et al*., 2003a). Several other 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).

3. USE OF ANTIMICROBIALS FOR *SALMONELLA* CONTROL

Antimicrobials are used as the main means of therapy for bacterial infection in human and veterinary medicine.

In the European Union, a veterinary medicinal product may be placed on the market in a particular Member State only if a marketing authorisation has been granted. There are different routes by which an antimicrobial may be granted a marketing authorisation in a particular Member State (Directive 2004/28/EC amending Directive 2001/82/EC); Member States shall implement this Directive by 30 October 2005 at the latest. A national authorisation may be granted following application to the competent authorities of that Member State. The product is evaluated in accordance with Directive 2004/28/EC amending Directive 2001/82/EC and the granted authorisation is valid nationally. That approval may later be extended to other Member States through a procedure of mutual recognition. A marketing authorisation may also be granted for all Member States following a centralised procedure in accordance with Regulation (EC 726/2004). In that case, applications are evaluated by the European Medicines Agency (EMEA, formerly called European Agency for Evaluation of Medicines) and decisions made by the Commission.

In all cases, decisions on authorisation are taken on basis of the scientific criteria of quality, safety and efficacy of the product concerned, following the guidance on pre-approval information for registration of new veterinary medicinal products for food producing animals with respect to antimicrobial resistance (Anonymous, 2004). Antimicrobials for use in food producing animals may only be dispensed after a veterinary prescription has been made out. The marketing authorisation is granted for specified conditions (indications). In authorisations granted in recent years, these conditions are mostly specific diseases or infectious agents. Older authorisations may have less specific indications such as “infections in poultry caused by organisms susceptible to X (the active substance)”.

If there is no authorised veterinary product in a Member State for a condition affecting food-producing species by way of exception the veterinarian responsible may, under his direct personal responsibility and in particular to avoid causing unacceptable suffering, prescribe for the animals concerned in a particular holding, a veterinary product authorised for use in another animal species or for another condition in the same species. If there is no such product on the market, a product authorised for human use in that Member State, or a veterinary medicinal product authorised in another Member State may be used (the “Cascade” principle laid down in article 11 of Directive 2001/82/EC). This only applies if the food safety aspect of the active

substances has been assessed and that it has not been listed as an unauthorised substance in annex IV of Regulation No 2377/90.

Administration of antimicrobials as veterinary medicinal products to poultry in commercial rearing is exclusively in the form of flock medication. The antimicrobial can be administered orally though water or feed, or as subcutaneous injections of newly hatched chicks. Dipping of eggs in antibiotic solutions, or injection of eggs, is also practised in some countries to control the vertical spread of certain bacterial infections; these practices are not included in the Marketing Authorisation in the EU. For all group medication, it is essential to ensure that the correct dose is delivered to all animals. In poultry, all medication must be given orally and metaphylactically, since individual treatment of sick birds is not possible, except occasionally in the case of small flocks or expensive breeding birds, especially turkeys (Bishop, 1998; Wray and Davies, 2002). Medicated feed can be used for long-term prevention of disease, and may be more cost-effective than water medication. *Salmonella* spp. rarely causes primary disease in poultry but may be associated with primary viral conditions, such as Turkey Rhinotracheitis virus, or conditions leading to intestinal damage, such as coccidiosis. Since diagnosis and confirmation of *Salmonella* spp. can take several days, empirical treatment will have normally begun before laboratory results are available. Where possible, treatment should be based on antimicrobial susceptibility testing (WHO, 2001). If there is evidence or reason to suspect presence of nalidixic acid resistant *Salmonella* spp., fluoroquinolones should not be used for any therapeutic purpose (ACMSF, 1999). Neomycin or colistin are good options for local enteric infections since neomycin and colistin resistance is uncommon in *Salmonella* spp in most countries (Bishop, 1998).

In poultry, problems related to the method of administration must also be taken into account, since the treatment of large groups of animals via feed or drinking water can lead to an unequal distribution of the drug, and therefore diminish the efficacy and increase the risk of appearance of resistance (Sumano *et al*., 2003).

Data on usage of antimicrobials for poultry in most countries are still limited. Furthermore, there appear to be significant differences in bioequivalence of different formulations of an antimicrobial class (Sumano *et al.*, 2001) which may well lead to differences in efficacy and promotion of resistance.

In the E.U. many products have been authorised to be used in poultry production; this include, spectinomycine, amoxicillin, tetracyclines, potentiated sulphonamides (e.g. thrimethoprim-sulphonamide) and fluoroquinolones. The objectives of antimicrobial therapy are to lower mortality, to shorten the duration of clinical illness, and to eliminate the organism from the intestine. Antimicrobials considered to be most effective for control of *Salmonella* infections in poultry include the fluoroquinolones, aminoglycosides and cephalosporins such as ceftiofur (Annex 2). Ceftiofur is not authorized for poultry in most of the EU but may be used under the 'Cascade' prescribing system.

There appears to be consensus that most antimicrobials do not alter the course of enteric disease, and in humans "conventional antimicrobials" (amoxicillin, chloramphenicol, neomycin, ampicillin, tetracycline, trimethoprim/sulphonamide combinations) appear to neither shorten nor prolong the faecal excretion of salmonellae, whereas fluoroquinolones have been shown to reduce the degree of faecal shedding (van Duijkeren and Houwers, 2000).

If antimicrobial treatment for *Salmonella* is used, the effectiveness of the treatment and the effect on resistance in remaining organisms should be monitored. It is possible for example for *Salmonella* spp. to acquire additional transferable resistances (eg. to trimethoprim) from commensal flora when selective antimicrobial pressure is applied. (Skold, 2001).

The use of antimicrobials in poultry production is different in different countries, depending on the current regulations. Notwithstanding these various regulations, scientific data has been published concerning the efficacy of using antimicrobials in different situations to control *Salmonella* spp. as followed:

3.1. Possible ways of using antimicrobials to control *Salmonella* spp. in poultry flocks

a. Treatment of clinically affected flocks

Although *Salmonella* spp. infections usually remains subclinical in poultry, except in the case of *S.* Gallinarum/Pullorum infections, there are occasions where the organism may be primarily or secondarily involved in disease. It is then necessary to either treat or slaughter affected flocks.

b. Medication of flocks infected with *Salmonella* spp. without clinical signs

Treatment may be used to reduce the animal prevalence within flocks which are already infected. Examples of this are the treatment of infected layer pullets prior to moving to laying houses, treatment of breeding birds to reduce the prevalence of infected eggs and chicks or, in some situations, treatment of meat birds prior to slaughter to reduce levels of contamination at slaughter.

c. Prevention of *Salmonella* spp. infection in animals

In the European Union, antimicrobials should not be used in prevention of *Salmonella* spp infection.

3.2. Breeding flocks

In most developed countries, elite and grandparent chicken breeding flocks will normally be maintained under a high level of biosecurity, such that no *Salmonella* infection of any serovar is tolerated. Strict controls on farm inputs, farm staff and intensive monitoring ensures that this situation is maintained. In rare instances where *Salmonella*, especially *S.* Enteritidis and *S.* Typhimurium (and other serovars in some countries) are detected, the flock and all related

hatching eggs are rapidly destroyed to ensure that no infection is spread further through the breeding pyramid.

The situation in elite and grandparent turkey breeding flocks is less clear since in many countries in the EU, they are not subject to the same level of statutory control therefore it is possible that infected breeding flocks may sometimes remain in production. One option in this situation is to take measures to limit the dissemination of *Salmonella* spp. whilst arrangements to replace breeding birds are made. In such cases treatment of breeding flocks with antimicrobial treatment could be applied to chicks at the hatchery.

The situation with duck production and other poultry is even less clear, but it is recognised that in some countries in the EU antimicrobial treatment is regularly used for *Salmonella* control in breeder production of ducks and other poultry.

At parent flock level, *Salmonella* infection occurs occasionally and will transmit to commercial progeny by vertical transmission or by cross-infection during hatching of eggs and processing of chicks. It may therefore occasionally be an option to use antimicrobials in the hatchery or for progeny as a preventive measure to limit the further distribution of *Salmonella* spp., but the efficacy is questionable. The inclusion of *S.* Virchow, *S.* Hadar and *S.* Infantis in the EU monitoring and control programmes (EU, 2003b) may require further interventions in situations where there is endemic contamination.

One short term strategy which can be used to salvage valuable genetic material from infected breeding flocks is to treat this flock to establish a new *Salmonella*-free flock. In this case breeding birds could be medicated with an authorised veterinary medicinal product (e.g. fluoroquinolones) and eggs taken during the period of treatment to a *Salmonella*-free hatchery where chicks are hatched, intensively tested for *Salmonella* spp. during rearing and then used to set up new *Salmonella*-free breeding flocks to restock what was previously a chronically infected poultry company (Köhler and Poppel, 1994).

3.3. Hatching Eggs

Numerous publications have demonstrated the spread of *Salmonella* spp. in hatcher incubators, even with *Salmonella* serovars which would not normally be considered to be truly vertically transmitted (Cox *et al*., 1990, Bailey *et al.*, 1998, Berrang *et al*., 1999). When a vertically transmitted invasive serovar such as *S.* Enteritidis is present then cross-infection of chicks may be even more dramatic (Davies *et al*., 1997). Good hatchery practices, including egg disinfection and the use of formaldehyde during hatching can reduce this spread but not eliminate it. It may therefore be the case that one infected breeding flock may result in widespread infection of commercial poultry flocks.

In some countries outside the EU, in an emergency situation, when eggs from the breeding flock cannot be removed from the hatchery and destroyed, antimicrobial treatment of eggs or chicks may be used. Non-invasive egg treatment by dipping in antimicrobials has been described but produced mixed results. Immersion in disinfectants has not been very successful in controlling *Salmonella* spp. (Jodas, 1992; Cox *et al*., 1998). Eggs may be dipped using pressure differential dipping (PDD) (Greenfield *et al*., 1975) or temperature differential dipping (TDD) (Saif, 1972, Saif and Nestor, 1973). These methods produced good results experimentally (Hafez *et al*., 1995) but, may be more variable when applied to full scale commercial organisations (Eckperigin *et al*., 1983, Wilding *et al*., 1993). In PDD, eggs are immersed in antimicrobial solution within an airtight pressure tank. The pressure is then lowered to 500 mb for 5-6 minutes using a vacuum pump, after which the tank is allowed to return to atmospheric pressure and eggs are left in the liquid for approximately 10 minutes. During the reduced pressure phase, air leaves the eggs through open pores and is partially replaced by antimicrobial solution as normal pressure is restored. In TDD, eggs are washed, rinsed and sanitised, pre-warmed to 37-38^{o}C for 3-6 hours then immersed in antimicrobial solution at 15-16^{o}C for 15 minutes. Air within the egg expands and is expelled from the egg during the higher temperature phase to be partially replaced by antimicrobial during cooling. Dipping is limited by the porosity of eggs and the stage of storage/lay, which also affects shell quality. It is a technique which was widely and successfully used in the USA to control *'arizonosis'* in turkeys (Mayeda *et al.*, 1978; Bagley, 1979; Bock *et al*., 1980; Reva, 1982). Antimicrobials which have been used are gentamicin, kanamycin, streptomycin or enrofloxacin either singly, or in combination (Wilding *et al.*, 1993, Kolahi and Keles, 2002). The technique has been used in situations where there is overwhelming *Salmonella* infection but is insufficiently efficacious for long term control and also is likely to result in the selection of resistant organisms (Nivas *et al*., 1975, Dubel *et al*., 1982).

Better results for egg treatment have been claimed for egg injection, which can either be done manually or through an automated system such as 'Inovoject'. Egg injection has been very successful for administration of viral vaccines (Gagic *et al*., 1999) and has been tried with less success for administration of competitive exclusion culture (Cox *et al*., 1992). In the USA, *in ovo* injection with fluoroquinolones, gentamicin or ceftiofur can be used to assist the control of *Salmonella* spp. (McReynolds *et al.*, 2000), so may be used to reduce the dissemination of infection from known infected breeding flocks. Treatment may not be 100% successful in eggs which are infected internally by vertical transmission (Bailey and Line, 2001) since the infection may be well established by the time egg injections are administered on transfer to hatchery incubators, at approximately 18 days in the case of chickens. Where antimicrobial treatment of purchased hatching eggs is suspected bioassays can be used to confirm this (Caldwell *et al*., 2000). In the EU, there is no Marketing Authorisation for the use of celtiofur and for *in ovo* injection to control *Salmonella* infection in poultry production.

3.4. Chick/Poult Medication: Hatchery

If antimicrobial medication is to be used for infected birds or as a preventive measure, then parenteral treatment is preferable for elimination of systemic *Salmonella* infection. This is normally only practical at the hatchery because of the availability of semi-automated injection facilities. Antimicrobials such as gentamicin, fluoroquinolones or appropriate cephalosporins, e.g. ceftiofur, may be conveniently mixed in the same injection as the routine Marek's Disease vaccine (Anonymous, 1999; Tanner, 2000). This however only allows a one dose course which may not completely eliminate infection in chicks from infected breeding flocks but may significantly reduce the chance of colonisation of chicks by *Salmonella* spp. from a contaminated hatchery. Mixing of vaccines with antimicrobials is not part of Marketing Authorisation in the E.U and the risks associated with this practice have not been evaluated.

3.5. Chick/Poult Medication: Farm

One option for treatment of chicks or poults which are likely to have been exposed to *Salmonella* spp. before hatching or at the hatchery is the preventive use of antimicrobials in water or in feed. The former is more common as young birds are more likely to drink at an early stage than to eat (Tanner, 2000), although early water intake may still be variable. A range of antimicrobials may be used preventatively for chicks (McMullin, 2001), especially those derived from young breeding flocks, and of these apramycin, spectinomycin, neomycin, amoxicillin, fluoroquinolones, potentiated sulphonamides and tetracyclines may reduce *Salmonella* infection. If chicks are already colonised by *Salmonella* before treatment it is unlikely that 100% clearance of infection will be achieved (Chadfield and Hinton, 2003). In most countries fluoroquinolones are used sparingly in line with prudent use initiatives, and would only be used for control of *Salmonella* spp. outbreaks as an urgent short term measure (SCVPH, 2003). In most countries fluoroquinolones are used sparingly in line with prudent use initiatives, and would only be used for control of *Salmonella* spp. as an urgent short term measure (SCVPH, 2003).

3.6. Pre-Slaughter Medication

There are anecdotal reports from some countries of the use of antimicrobials during the later stages of rearing of meat birds without clinical signs, particularly turkeys, to achieve low levels of *Salmonella* spp. on pre-slaughter check tests. It is thought that this form of treatment, using fluoroquinolones, may have led to a reduced susceptibility in certain countries and poultry meat species, but there is no published evidence for this. This use to reduce *Salmonella* spp. excretion is not part of Marketing Authorisations in the E.U. and is considered not prudent use.

3.7. Egg production

In some circumstances, antimicrobials, particularly fluoroquinolones followed by competitive exclusion treatment are used in an attempt to eliminate *Salmonella* infection without clinical signs in commercial laying birds during

rear so that flocks moved to laying houses may be *Salmonella*-free during lay. Although this may reduce *Salmonella* levels for a time it is unlikely to be totally successful (Seo *et al.*, 2000b) and poses a risk of establishing a permanent infection cycle in the laying house, if it is not already contaminated from previous flocks (Davies *et al.*, 2003) as well as the development of resistance and the introduction of resistant organism in the layers that might be transferred to the humans.

Although chlortetracycline or oxytetracycline may occasionally be used for therapeutic purposes they are unlikely to have much effect on *Salmonella* carriage, unless a resistant *Salmonella* spp. is present, when their use may favour colonisation and dissemination of the organism as well as resistance.

4. ADVANTAGES AND DISADVANTAGES OF THE USE OF ANTIMICROBIALS FOR CONTROL OF *SALMONELLA*

The advantages and disadvantages are listed irrespectively of their practicability or current application.

4.1. Advantages

Control of *Salmonella* spp.

The use of antimicrobials, on the basis of a veterinary prescription and by respecting the withdrawal time, is one of the means to control bacterial infections in food animals. During a *Salmonella* infection in a poultry flock, the administration of approved antimicrobials may reduce the dissemination of the bacteria to other birds, to the environment and to food products. .

Antimicrobial therapy may be used while waiting for the confirmation of *Salmonella* spp. in a breeding flock, as long as samples are taken before treatment is begun. This will limit the spread of infection during the isolation and serotyping process before the flock is confirmed as infected and further action is taken.

Strategic use of antimicrobials may be an advantage in the initial stages of *Salmonella* control programmes covering those serovars for which vaccines are not available as the chosen antimicrobial may be effective against all susceptible strains, and not only *S.* Enteritidis and *S.* Typhimurium..

Rapid implementation

Antimicrobial treatment can be implemented rapidly within flocks of high prevalence (see above). The effect may not last long enough for eradication of *Salmonella* spp. (Redmann *et al.*, 1989) but such treatment may be an interim measure.

In an emergency situation, when eggs from the breeding flock cannot be removed from the hatchery and destroyed, antimicrobial treatment of eggs or chicks may be considered as a short-term measure.

Antimicrobials and environmental contamination

The application of antimicrobials may have an effect on the shedding of *Salmonella* spp. and on the number of bacteria present in the gastro-intestinal tract and thus may reduce the load of *Salmonella* spp. that contributes to the environmental contamination *via* faeces. This makes cleaning and disinfection , prior to restocking, more likely to be effective (Reynolds *et al.*, 1997; Davies *and Breslin*, 2003b). Manure and other waste from the house are also less likely to be heavily contaminated.

Combination with CE or probiotics

The effect of antimicrobial therapy can be improved if it is followed by competitive exclusion treatment to help restore the disrupted enteric flora and combined with moving the birds to new *Salmonella*-free premises.
Probiotics, i.e. single or a few well defined strains (lactic acid bacteria, *Bacillus* spp. or yeasts) are also used to restore the flora of the intestinal tract after antimicrobial treatment. Probiotics aim also to protect the intestinal microflora against pathogenic bacteria, some having bactericidal activity also against salmonella. They are not used as the main or only measure against pathogens but as accompanying measure.
Combination of antimicrobials with these strategies may be an option where no antagonism of the antimicrobial against the efficacy of the other control measure is to be expected. CE as well as the application of single probiotic strains includes mainly lactic acid bacteria like lactobacilli and enterococci. Lactobacilli, especially strains from the *L. casei* group like *L. rhamnosus* are rarely resistant to the antimicrobials of concern. They normally exhibit susceptibility to aminoglycosides (gentamicin), quinolones (ciprofloxacin/enrofloxacin) and cephalosporins (Klein, 1998). Those strains can therefore not be used in combination with antimicrobials. Enterococci, especially probiotic strains of *E. faecium* are often resistant to cephalosporins and aminoglycosides (gentamicin), but are susceptible against quinolones (ciprofloxacin) (Klein, 1998). Those strains can be applied in combination depending on the antimicrobials used and the resistance pattern of the specific probiotic strain.
The possibility of selecting for antimicrobial resistance through the use of probiotic strains must be excluded.. However, so far only limited data are available in regard to this problem (Klein *et al*., 2000).
Evaluation of the effect of antimicrobial application on the microbes of Competitive Exclusion (CE) is needed.

Control of other pathogens

The chosen antimicrobial may also have an effect on other microorganisms in avian bacterial diseases such as air sacculitis or yolk sac infections in 1-day old chicks caused by bacteria such as *E.coli*.

4.2. Disadvantages

The adverse effects of use of antimicrobials can be divided in two categories:

- Effects related to the antimicrobial nature of the substances such as development and spread of antimicrobial resistance, and decreased colonisation resistance
- Effects related to potential toxicological effects resulting from the exposure of the target species, of the consumer, of those who work with the substance, or of the environment.

The potentials hazards associated with toxicological aspects of antimicrobials use will not be discussed further.

Another possible disadvantage is that chicks derived from antimicrobial treated eggs or from breeding flocks currently undergoing treatment may not be receptive to live *Salmonella* vaccines or competitive exclusion culture (McReynolds *et al*., 2000).

4.2.1. Effects of the use of antimicrobials on resistant bacteria or genes and their transfer within and between ecosystems

The general basis for emergence and spread of resistance has been detailed in a number of opinions and reports (e.g. SSC 1999; SCVPH 2003; FAO/OIE/WHO 2003; EC, 2004). When an animal is exposed to an antimicrobial, the survival and multiplication of resistant pathogens and commensals will be favoured (selection). The resistant bacteria, or resistance genes, may then spread to other animals in the same group or flock, to animals of the same species at other farms, or to other animal species. Further, such transfer can also occur to human either through direct contact, or through consumption or handling of contaminated food.

The use of a specific antimicrobial may favour not only the dissemination of the corresponding resistance gene, but also that of other resistance genes located on the same genetic element (co-selection). Genes conveying resistance to heavy metals or virulence factors may also reside on transposons or plasmids together with antibiotic resistance genes, thus offering further possibilities for co-selection (McHugh *et al*., 1975; Summers 2002).

The link between exposure to antimicrobials and selection of resistance has been demonstrated in a number of studies (see for example SSC, 1999; FAO/OIE/WHO, 2003; EC, 2004). However, the dynamics of emergence and

spread of resistant bacteria within and between different ecological compartments is complex and also depends on factors related to the spread of infections, such as hygiene (including food hygiene), contact rate and clonal expansion.

In the opinion issued by the Scientific Steering Committee, it was concluded that the increasing prevalence of antimicrobial resistance among bacteria has serious implications for human and animal health (SSC, 1999).

4.2.1.1. *Salmonella* spp.

Emergence and spread of resistance

Experimental evidence of *in vivo* transfer of multiple resistance determinants from E*scherichia coli* to *Salmonella* spp. has been reported (Smith and Tucker, 1975; Gast and Stephens, 1986; Gast *et al.*, 1988). Once resistance has emerged or been introduced, administration of antimicrobials leads to an increased shedding of bacteria (Latour and Barnum, 1981; Kobland *et al.*, 1987; Manning *et al.*, 1994). This may affect the degree of contamination of food products (Gast *et al.*, 1988).

Use of quinolones in poultry, especially turkeys, has led to the emergence of *Salmonella* spp. which are resistant to older style quinolone antimicrobials, such as nalidixic acid or flumequine, and with reduced susceptibility to fluoroquinolones (Davies *et al.*, 1999; Rabsch *et al.*, 2001; Jones *et al.*, 2002; Maran, 2002). Resistance problems are worse in countries where there is more use of fluoroquinolones (Hakanen *et al.*, 2001; Mammina *et al.*, 2002; Mølbak *et al.*, 2002; Usera *et al.*, 2002; Antunes *et al.*, 2003). Emergence of resistance following use of gentamicin specifically to control *Salmonella* infections in turkeys has been reported (Helmuth and Protz, 1997).

The spread of antimicrobial resistant *Salmonella* spp. from animals to humans *via* the food chain, or through direct contact, is well documented (SSC, 1999; SCVPH, 2003; FAO/OIE/WHO, 2003). In many studies, the occurrence of such resistant strains in animals or food of animal origin have been directly linked to the use of antimicrobials in primary production (Holmberg *et al.*, 1984; Spika *et al.*, 1987; Dunne *et al.*, 2000; Fey *et al.*, 2000). The picture is, very complex and may not be equally valid for all serovars and phage types (Threlfall *et al.*, 2003). The interpretation of national or regional data is further complicated by the global nature of food trade.

Adverse effects on public health

The main adverse effect of resistance in *Salmonella* spp. is the reduced effectiveness of antimicrobials used for therapy of people (SCVPH, 2003a; FAO/OIE/WHO, 2003). In many cases, infections caused by non-typhoidal *Salmonella* may resolve without treatment. In particular in children, in the elderly and in immunocompromised patients, however, the disease can be

severe and effective antimicrobial treatment can be life saving. Another potential consequence of resistance is an increased incidence of salmonellosis in patients who, for unrelated reasons, are taking antimicrobials (Barza and Travers, 2002).

The use of fluoroquinolones and cephalosporins in animal production is particularly controversial, since these antimicrobials are important for the therapy of human systemic bacterial infections (FAO/OIE/WHO, 2003). Use of fluoroquinolones in poultry is seen as a major factor in emergence of resistant *Salmonella* spp. in humans (Angulo *et al.*, 2000; Threlfall *et al.*, 2000; Hakanen *et al.*, 2001) and is less of a problem in countries where such usage has been restricted (Pedersen *et al.*, 2002). Similarly, the use of ceftiofur for animals in the USA has come under increased scrutiny as a factor contributing to the emergence and spread of multidrug-resistant cephalosporin-resistant *Salmonella* (Winokur *et al*., 2000, Dunne *et al*., 2000).

4.2.1.2. *Campylobacter spp.*

Emergence and spread of resistance

Exposure of chickens to fluoroquinolones at authorised doses is associated with a rapid emergence of resistance in *Campylobacter* spp. both in individual birds (Van Boven *et al.*, 2003) and among groups of animals (Jacobs-Reitsma *et al.*, 1994, McDermott *et al.*, 2003).

In several EU countries, the prevalence of resistance to fluoroquinolones among *Campylobacter* spp. from chickens has increased after the authorisation, and assumed use of fluoroquinolones in food-animals (Endtz *et al.*, 1991; Velazquez *et al.*, 1995; Threlfall *et al.*, 1999b), and this increase was paralleled by an increase among isolates from people. An exception to this is the Swedish situation, where *Campylobacter jejuni* isolated from chickens have remained almost uniformely susceptible to quinolones for more than 10 years after the authorisation of fluoroquinolones for use in poultry (SVARM, 2002). In that country, fluoroquinolones are used in poultry production as a first choice for treatment of *E. coli* septicaemia but as clinical problems are rare, the use is very limited (SVARM, 2000). Similar observations have also been made from Norway (NORM-VET, 2002). In Norway in 2001, the prevalence of quinolone resistance among *Campylobacter* isolates from domestic poultry and from domestically acquired cases of campylobacteriosis in humans was low (2.7% vs. 7%), as opposed to a high prevalence of quinolone resistance in isolates from imported human cases (60%) (Kruse *et al*, 2002; SCVPH, 2003;). In Australia, where fluoroquinolones have not been authorised for use in animals, indigenous fluoroquinolone resistant *Campylobacter* are not seen in humans (Unicomb, 2003).

Adverse effects on public health

Campylobacter jejuni/coli are food-borne pathogens, and one important source of human infections is poultry. The adverse effect of resistance in *Campylobacter* is reduced effectiveness of specific antimicrobials such as erythromycin and ciprofloxacin which may be used for therapy in cases of human infection. (SCVPH, 2003a; FAO/OIE/WHO, 2003). There is increasing published evidence on adverse effects on public health (FAO/OIE/WHO, 2003).

4.2.1.3. Avian pathogens (*E. coli*)

Emergence and spread of resistance

Resistance among clinical isolates of poultry *E. coli* can be high and multiple resistance is common, as demonstrated in studies from Spain and the US (Blanco *et al.*, 1997; Bass *et al.*, 1999). In a collection of strains isolated from various types of poultry in the US, 63% of the strains were found to harbour class 1 type integrons, mostly located in a transposon related to Tn*21* (Bass *et al.*, 1999). In the US, an increase in resistance to fluoroquinolones among avian pathogenic *E. coli* has been reported (White *et al.*, 2000).

Adverse effect on animal health

Diseases resulting from infections, such as septicaemia, air sacculitis and subcutaneous infections can cause high morbidity and mortality in chickens and turkeys and may have a significant economic impact on poultry production. There are a limited number of antimicrobials available for treatment of poultry, and the frequent occurrence of multiple resistances, including resistance to fluoroquinolones, is a cause for concern. Any increase in the prevalence of resistance to drugs still effective for treatment can have an adverse effect on animal health.

4.2.1.4. Commensal bacteria - horizontal transfer of resistance genes

Emergence and spread of resistance

Exposure of animals to antibiotics is associated with an increased prevalence of resistance among bacteria of the normal flora (Hinton *et al.*, 1986; SSC, 1999). Resistance may persist for a prolonged time after, or even without exposure to antibiotics (Hinton *et al.*, 1986; Chaslus–Dancla *et al.*, 1987).

It has been suggested that, in the normal human population, most resistant enterobacteria in faeces come from contaminated food (Corpet, 1988). During the passage through the intestine, these transient bacteria may transfer their resistance genes to bacteria better adapted to the host (humans) (Oppegaard *et al.*, 2001, Moubareck *et al.*, 2003. Exchange of resistance genes between bacteria from different sources has also been demonstrated in water, soil, on

kitchen towels, on cutting boards, and on the surface of food (Kruse and Sørum, 1994).

Several examples of the spread of resistance genes from bacteria colonising animals to human microflora or pathogens have been documented (Levy *et al.*, 1976; Chaslus-Dancla *et al*, 1986; Hummel *et al*, 1986; Wray *et al*, 1986; Threlfall *et al*, 1986, Chaslus-Dancla *et al*, 1989; Salauze *et al*, 1990; Chaslus-Dancla *et al*, 1991; Hunter *et al*, 1993; Hunter *et al*, 1994; Tschäpe, 1994). Further, the finding of identical, or nearly identical, gene sequences in anaerobic bacteria of human origin compared to those of bovine origin indicate that transmission of genes has occurred in nature between bacteria normally colonising different hosts. (Nikolich *et al*., 1994)

Adverse effects on public health

There is evidence to support a flow of resistance genes between commensal and pathogenic bacteria, within and between different ecological compartments (Courvalin, 1994; SSC, 1999). Resistance genes may be transferred from commensals to pathogens of the exposed animal species, but also to commensals and pathogens of other animal species and people. Similarly, resistance genes originating from bacteria normally colonising human can be transferred to animal commensals or pathogens. The relative contributions of the many factors involved in this exchange are, however, still not well understood and it is difficult to predict the epidemiology based on patterns of antimicrobials used alone (SSC, 1999). The adverse effect of any transfer of resistance to pathogens of animal or human is the loss of effectiveness of antimicrobials for treatment in animal and human diseases.

4.2.2. Other effects

The protective effect of the normal microflora against *Salmonella* infection was demonstrated by Nurmi and Rantala in the early 70s (Nurmi and Rantala, 1973). All exposure of the normal flora to antimicrobials will disturb its balance (Corpet, 1996). This may lead to a decreased resistance against colonisation with *Salmonella* spp. and *Campylobacter* spp. that are resistant to the antimicrobial applied by lowering the infectious dose needed for successful colonisation. This would increase the likelihood of a poultry flock getting colonised by *Salmonella* spp., with an accompanying increased probability of food products getting contaminated.

Treatment with antimicrobials that have activity against *Salmonella* spp. will reduce the number of *Salmonella* organisms shed by the individual animal, and the prevalence within a flock, but will in many cases not eliminate the infection completely. This may interfere with diagnostics in control programmes (see section 5).

During treatment with an antimicrobial, the proportion of resistant bacteria shed to the environment will increase. Manure and waste from the house is likely to

be contaminated with resistant strains, and depending on the degradability of the antimicrobial used, also with antimicrobials. Resistant bacteria may survive for prolonged periods of time in slurry (Hinton and Linton, 1982). Resistance genes originating from animal waste have been detected in groundwater underlying swine production facilities, both in commensals and in environmental bacteria (Chee-Sanford *et al*., 2001). Contaminated soil, groundwater and waterways may facilitate the spread of bacteria carrying resistance traits, and are a potential source of antimicrobial resistance in the food chain.

4.2.3. Disadvantages in relation to different types of flocks

Any use of antimicrobials will exert a selective pressure on microbial populations and contribute to emergence and spread of resistance in zoonotic bacteria such as *Salmonella* spp. and *Campylobacter*spp., in animal pathogens (eg. *E. coli*) and in commensal bacteria. The hazard as such is the same for different types of flock or production systems. The risk for public or animal health, however, will depend on a number of factors such as bacterial species, antimicrobial substance, amounts used, type of flock, number of animals or flocks exposed and hygiene at different stages of the primary production as well as during processing. If meat birds are treated close to slaughter the risk of large numbers of resistant *Campylobacter* spp. and commensal bacteria entering the food chain on individual carcases from the flock will be greatest but if resistant organisms are selected by antimicrobial treatment of breeding flocks and are capable of vertical or hatchery transmission (eg. *Salmonella* spp.) a larger number of commercial flocks may become infected but with a lower number of resistant organisms per carcase. Flocks which are clinically infected with *Salmonella* spp. will harbour larger numbers of organisms than subclinically infected flocks. This presents a greater risk of selection of resistant mutants, however the greatest public health risk is when a resistant strain is already present, since use of ineffective antimicrobials will promote its multiplication and spread.

5. QUALITATIVE ASSESSMENT OF THE RISKS TO HUMAN HEALTH THAT COULD RESULT FROM THE USE OF ANTIMICROBIALS

A generally accepted definition of risk is given by Ahl *et al*. (1993) as the likelihood and magnitude of the occurrence of an adverse event. The risks to human health due to the use of antimicrobials against *Salmonella* spp., as far as the possibility of resistance transfer is concerned, take into account the following:

- **emergence** of resistance among bacterial population, considering the target species (*Salmonella* spp.), other zoonotic agents (Campylobacter spp.) and commensals bacteria;
- **spread** of resistance within the treated flock;
- **diffusion** of resistance to the progeny of the treated flock;
- **transfer** of resistance to humans via contaminated food (meat and eggs) and its possible outcomes.

In each of these steps many different variables must be considered, among which the most important are:

Step 1: emergence

a) Use of antimicrobials (substance, dosage, duration of treatment, way of administration);
b) type of resistance (transmissible – e.g. plasmid-mediated or chromosomal – mutational);
c) type of microorganisms involved.

Step 2: spread within the flock

a) Prevalence of infected animals;
b) average number of bacteria per animal (sick, asymptomatic, non infected).

Step 3: diffusion

a) Kind of flock, and level in the production pyramid;
b) infection prevalence within the flock;
c) transmission rate of the involved microorganism;

Step 4: transfer to humans

a) likelihood of transmission of resistant strains to food products (carcasses, eggs);
b) factors influencing food contamination (processing, storage, cooking, etc.);
c) consumption data;
d) factors influencing colonization and possible outcomes.

With these points in mind, a table has been prepared showing the qualitative risk regarding emergence and spread of resistance (step 1 and 2): 1) when antimicrobials are used for the prevention of *Salmonella* infection in animals, 2) for the treatment of flocks infected with *Salmonella* spp. without clinical signs, and: 3) for the treatment of clinically-infected flocks.
For each of the three purposes the table shows the likelihood of the emergence and spread of resistance in relation to antimicrobials promoting chromosomal (mutational) resistance, e.g, to quinolone antimicrobials, and those promoting transmissible (ie, plasmid-mediated) resistance.
The results must then be adjusted to practical cases, in particular considering the prevalence of infected animals (step 2a). The possibility of spreading resistance to *Campylobacter* spp. has also been included. It should be realised that the values shown are subjective and can be changed by a rare, unpredictable event.
For each of these scenarios the probability of the emergence or spread of resistance in a flock has been estimated from negligible (+) to low (++), medium (+++) or high (++++), based on the model of Moutou *et al.* (2001).

The criteria used to identify the level of risk have been based on mutation rates (in relation to chromosomal resistance) (Billington and Gillespie, 2000), for transmissible resistance, to rates of transfer for plasmids commonly associated with resistance to aminoglycosides in Gram-negative bacteria (Anderson and Threlfall, 1974), and to the

quantity and duration of exposure to the antimicrobials under the different usage criteria as listed above.

For *Salmonella* spp, this qualitative assessment of the development and spread of resistance may then be coupled with different types of poultry production, based on the pyramidal structure shown in figure 1, all of which will give different probabilities of resistant strains spreading or becoming established in flocks. Considering the magnitude of transmission throughout the pyramidal structure previously described, irrespectively of any other factor involved, it appears that a great grandparent can disseminate a resistant strain till the basis of the pyramid, theoretically leading to the contamination of more than 150.000 animals. In the case of grandparent or a parent this number will be much lower, whereas the likelihood of diffusion to the progeny in broiler flocks or in commercial layers will be 0. This distribution can be mathematically described using an exponential function (Annex 3). Accordingly, once the risk of emergence and spread of resistance has been assessed, the risk of diffusion to the progeny can be calculated, considering that in parents the risk of diffusion is very close to the one of broilers (close to 0), in grandparents is about three times more, and in great grandparents is 100 times more. In this calculation other variables must be taken into account, as the prevalence of infected animals at different stages and other factors able to interphere on transmission (biosecurity, vaccination, etc.).Table 4 may then be used to estimate the probability of the dissemination of resistant strains associated with antimicrobial usage in the different production systems, considering that the grading reported in the table must be adjusted with the probability of diffusion, and must be adapted to different epidemiological situations, particularly taking into account the prevalence within and among flocks.

In terms of human health, the threat to public health caused by the dissemination of resistant strains of *Salmonella* spp. is directly related to the possibility of such strains entering the food chain. A particularly serious threat comes in the dissemination of resistant strains through shell eggs. Any measure whereby the use of antimicrobials promotes the appearance and dissemination of resistant strains in the primary breeding flock or hatchery must be regarded as a very high risk and should be actively discouraged by whatever means possible. Vertically transmitted strains will also present an increased threat because of their ability to contaminate the contents of table eggs. In contrast for *Campylobacter* spp., where the normal means of contamination is through commercial meat birds, then the use of antimicrobials in production flocks poses a significantly higher risk to public health than treatment of breeding flocks.

Table 4 Probability of emergence and spread of resistance

Treatment	Clinically-infected flocks		Prevention of *Salmonella* infection		Infected flocks without clinical signs	
Probability of:	1*	2*	1*	2*	1*	2*
Development of resistance (mutational)	++	+	+++	+	++++	+
Acquisition of resistance (transmissible)	+	+++	+	++	+	+++
Selection of resistant strains	+++	+++	+++	+++	++++	++++
Spread of resistance strains	++++	++++	+++	+++	+++	+++
Spread of resistance genes:						
To pathogens (*Salmonella* spp.)	+	++++	+	++	+	+++
To pathogens (*Campylobacter* spp.)	+	++	+	++	+	++
To non-pathogens (commensals)	+	++++	+	++	+	+++
Cross-resistance:						
To related antimicrobials	++++	++++	++++	++++	++++	+++
To unrelated antimicrobials	+	++++	+	++	+	++

Treatment with:1 Antimicrobials promoting chromosomal resistance (e.g. quinolones). 2* Antimicrobials promoting transmissible resistance (e.g. aminoglycosides).+ indicates whether or not there is a probability; the level of probability is graded as negligible (+), low (++), medium (+++) or high (++++)*

6. INTERFERENCE OF USE OF ANTIMICROBIALS WITH THE SUCCESSFUL IMPLEMENTATION OF A CONTROL PROGRAMME

Salmonella monitoring in poultry is based on flock periodic testing by means of different methods, with the aim of detecting positive flocks, assessing the prevalence of infected flocks or detecting changes in prevalence. The most frequently used methods are bacteriological and serological ones.

6.1. Interference with bacteriological testing

Bacteriological testing can be performed on animal samples (faeces, cloacal swabs, organs, eggs) or on environmental samples. In the first case different sampling schemes can be used, depending on the aim of the monitoring. A sampling scheme aimed at assessing the prevalence of infected flocks in a country or area, must take into account:

- the expected prevalence of infected flocks;
- the expected prevalence of positive (or shedder) birds within the flock;
- the desired level of accuracy and confidence limits.

By environmental monitoring it is possible to assess the prevalence of contaminated flocks, and sampling schemes must take into account the expected prevalence of contaminated flocks and the desired level of accuracy and confidence limits.

In general in the case of animal testing, the lower the flock prevalence, the higher the number of samples to be taken. In these cases in fact sampling schemes are not designed in order to assess the prevalence, but to find at least one positive sample if the prevalence is below a certain level. Generally, 60 single samples are taken, in order to detect within a flock prevalence of 5% or more. If faecal samples or cloacal swabs are taken, the flock prevalence corresponds to the percentage of animals shedding *Salmonella* at the time of sampling.

In the case of antimicrobials use in a situation in which the prevalence is low (e.g. breeders), the treatment could lower the prevalence of infected flocks and within the flock prevalence (i.e. the number of animals excreting the bacteria) below the detection limit of routinely used sampling schemes, giving rise to a monitoring result in which negativity does not correspond to the real infection status of flocks.

The same situation could be true if environmental sampling is used (e.g. in layers, where sampling of egg belts and dust can be effective in detecting positive flocks), since antimicrobial treatments diminish environmental contamination and can have an inhibitory effect on bacteriological testing, and so false negative results could be obtained.

In these cases, different sampling schemes should be adopted, in order to take into account the possibility that the number of animals excreting *Salmonella* in faeces is very low, and consequently using an expected prevalence below 1%. It should also be considered that in some cases the flock remains negative for some weeks after the treatment, but can experience bacteriological relapse afterwards, as it has been demonstrated in chickens (Humbert *et al*., 1997) and in humans (Pichler *et al.*, 1987), and so bacteriological monitoring should be repeated for a certain time after the antimicrobial treatment.

In case of hatching eggs monitoring in breeders, it should be considered that antimicrobial treatment can drastically reduce or eliminate the transmission of *Salmonellae* via eggs, and so monitoring of dead in shell or cull chicks or meconium may no longer be representative of the infection status of the flock of origin.

In situations in which the prevalence of infection among and within flocks is expected to be high -at least 10% after treatment- the use of antimicrobials should not hamper the application of monitoring programmes. In cases where sampling schemes are aimed at assessing the prevalence of positive flocks, and the number of samples necessary to assess the prevalence when the expected prevalence is for example 50, 40 or 30% is sufficient also in cases of lower expected prevalences (50% is the worst case, in which the maximum number of samples is required for estimates of flock prevalences).

In any case, a certain length of time must be ensured between treatment and bacteriological monitoring, in order to avoid any interference of the drug with bacteriological examination.

6.2. Interference with serological testing

Serological monitoring is based on the same statistical criteria as exposed for bacteriological monitoring, with the difference that the prevalence of reactors is assessed, instead of the prevalence of animals shedding *Salmonella*, as in the case of faecal samples or cloacal swabs bacteriological examination.

Antimicrobial treatment, as previously stated, decreases *Salmonella* excretion, and therefore lowers the risk of transmission, being infection dose-dependent. The number of reactors in a flock could consequently be lower than what it would be without treatment, and so the same considerations expressed for bacteriological testing could be valid, with the possibility of not identifying correctly infected flocks if the prevalence is low, and probably not a big impact in case of high prevalences. Some studies have shown a reduction in antibody levels in infected birds after treatment, which is likely to be due to reduced ongoing stimulation of antibody production (Desmidt *et al.*, 1992; Goren, 1992; Reynolds *et al.*, 1997).

Despite this, serological monitoring where a suitable test is available, is more likely to detect flocks infected with the target serovar when infected animals are treated and become bacteriological negative, since antibodies will be present for a longer period of time.

7. CONCLUSIONS

Salmonella spp. is widespread in poultry production in Europe. Prevalences vary considerably depending on country and type of production. Prevalences are lowest at the top of the production pyramid, i.e., the breeder stages.

Poultry meat and eggs represent an important source of human infection with *Salmonella* spp.

S. Enteritidis and *S.* Typhimurium are the most commonly reported serovars isolated from poultry, poultry meat products and human cases of salmonellosis.

Antimicrobial resistance in *Salmonella* spp. and other bacteria is an increasing public health concern.

The consequences of resistance to certain antimicrobials, especially fluoroquinolones and cephalosporins, are of particular concern, since these are critically important for therapy of human systemic bacterial infections.

The risk to public health from the selection of resistant organisms depends on the likelihood of this event for a particular bacterium, the behaviour and prevalence of the bacteria, the antimicrobial in question, the type of resistance (transmissible or not, possibility of cross-resistance and co-selection), and type and stage of poultry production.

Advantages and disadvantages of the use of antimicrobials in the framework of programmes to control *Salmonella* spp. in poultry

- The advantages of antimicrobials used in poultry and listed below must be balanced against the risks associated with the development, selection and spread of antimicrobial resistance and the impact of such resistant organisms in public health.

- The remarks on antimicrobial usage for the control of *Salmonella* spp. in poultry, discussed below, apply to all the major commercial poultry species.

- The most commonly used antimicrobials target all serovars of *Salmonella* spp. However, all the strains are not susceptible to all existing antimicrobials.

<u>General advantages and disadvantages related to all types of poultry</u>

- Any use of antimicrobials in poultry will increase the risk of emergence and spread of resistance in zoonotic bacteria such as *Salmonella* spp. and *Campylobacter* spp., as well as in animal pathogens and commensal bacteria. However, on the rare occasions when *Salmonella* spp. causes clinical infections in poultry, antimicrobials may be useful in reducing morbidity and mortality.

- The use of antimicrobials is never totally effective for the control of *Salmonella* spp. because it is not possible to eliminate all the organisms from an infected flock. However, antimicrobial use may reduce the within-flock prevalence of *Salmonella* infection and the level of excretion, and reduce environmental contamination. Thereby, the likelihood of spread to other flocks may be reduced and may limit the vertical transmission of *Salmonella* spp.

- The use of antimicrobials in poultry production can be implemented rapidly within flocks, thereby avoiding a rapid spread of infection if the bacteria are susceptible to the antimicrobial in question.

Breeder flocks

- Valuable genetic material may be salvaged from infected breeding flocks through the use of antimicrobials to provide *Salmonella*- free eggs in order to establish a new *Salmonella*-free flock. In breeder flocks the risk of dissemination of residual *Salmonella* spp., including resistant strains, through the production pyramid is high, compromising any potential advantage of treatment.

- If antimicrobials are used in breeding flocks or their immediate descendants there is a risk that any resistant bacteria which are selected may be disseminated to multiple flocks via contamination of hatching eggs and the hatchery environment.

Laying hens

- No specific advantages were identified in the case of laying hens. Some laying flocks may be persistently infected with *Salmonella* spp. so antimicrobial treatment presents a risk of maintaining a permanent infection cycle in the laying house as well as promoting the development, selection and dissemination of resistance.

Meat producing flocks

- No specific advantages were identified in the case of meat producing flocks. If infected broiler flocks are not depopulated, antimicrobials are incidentally used as a short term measure for broiler chicks which have originated from an infected parent flock or contaminated hatchery to limit the extent of subsequent infection. Antimicrobial treatment of meat producing birds increases the risk of carcase contamination with resistant *Salmonella* spp.and *Campylobacter* spp. as well as resistant commensal bacteria, which may also transfer resistance genes to other bacteria.

Risks associated with the use of antimicrobials to control *Salmonella* in poultry

- The likelihood of the development or acquisition of antimicrobial resistance in clinically infected flocks following the use of antimicrobials at therapeutic levels is considered to be lower than when sub-therapeutic levels or long-term treatment of antimicrobials are used, either for treatment or for the prevention of *Salmonella* spp. infection or for the medication of flocks without clinical signs.

- Should antimicrobial resistance be already present, develop or be acquired, then the use of antimicrobials for the treatment of clinically infected flocks, for the prevention of *Salmonella* infection, or for the treatment of infected flocks without clinical signs, will enhance the selection and spread of resistant bacterial strains throughout the production pyramid.

For the treatment of clinically affected flocks

- Clinically infected flocks, which have been treated with antimicrobials, are still considered contaminated with *Salmonella* spp.

For the prevention of *Salmonella* infection and for the treatment of infected flocks without clinical signs:

- Breeders: Use of antimicrobials in breeding flocks presents a risk of generation and wide dissemination of resistant organisms through the breeding pyramid.
- Laying flocks: Use of antimicrobials in commercial laying flocks presents a risk of generation of resistant organisms which may contaminate eggs and persist in the laying house to infect consecutive flocks of birds.
- Broiler flocks: Use of antimicrobials in broiler flocks presents a risk of generation of resistant organisms, including *Salmonella* spp. and *Campylobacter* spp., which may contaminate carcases and poultry meat.
- Turkeys and ducks: The principals of antimicrobial use in turkeys and ducks are the same as those for chickens; however in turkeys there are longer grow-out periods (brooding stage) which may allow for further dissemination of resistance and increase the frequency of medication of flocks for therapeutic purposes. The prevalence of *Salmonella* spp. in turkey and duck production may also be higher so exposure to therapeutic antimicrobials could provide a greater risk of generating resistance.

Aspects that may jeopardize a successful implementation of a programme to control *Salmonella* spp.

- There is a danger that antimicrobial treatment may be used as a substitute for good hygiene and biosecurity and so perpetuate the persistence of *Salmonella* spp. infection in consecutive poultry flocks, which is less likely if infected flocks were depopulated.
- Antimicrobial therapy can reduce the carriage and excretion of *Salmonella* spp. below the level of detection thereby reducing the diagnostic sensitivity of monitoring programs, and so may interfere with the detection or confirmation of infection.
- The misuse of antimicrobials may compromise the effectiveness of live bacterial vaccines, competitive exclusion cultures and probiotics.

In general, from a food safety/public health viewpoint, using antimicrobials to control *Salmonella* spp. in poultry has little justification. Any use in exceptional circumstances

on animal health and welfare grounds must recognize the consequences for public health.

8. RECOMMENDATIONS

The use of antimicrobials for *Salmonella* control in poultry should be discouraged due to public health risks associated with development, selection and spread of resistance. Their use should be subject to formally defined conditions that would ensure protection of public health. Such use must be fully approved in advance and recorded by the competent authority.

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GLOSSARY

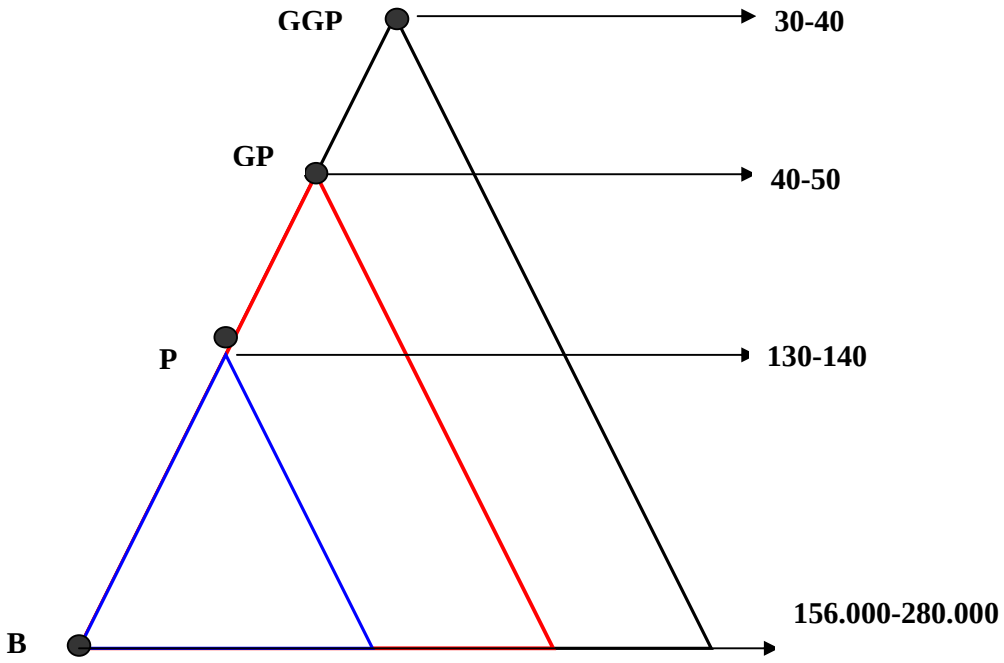
Definitions (based on EC 1999)

Antibiotic:	a substance, produced by or derived from a micro-organism, which inhibits the growth of or destroys other micro-organisms.
Antimicrobial:	a drug, not a disinfectant, which, at low concentrates, exerts an action against microbial pathogens and exhibits selective toxicity towards them.
Asymptomatic:	not showing any symptoms of disease, whether a disease is present or not.

Empirical treatment:	management of diseases, such as dung treatment, based on experience or observation rather than on specific laboratory investigations.
Prebiotic:	a non-digestible feed or food ingredient which passes through the small intestine and promotes the growth of autochthonous or inoculated probiotic bacteria.
Preventive antimicrobial therapy:	This includes short term prophylaxis but may involve more extended periods of treatment when a prolonged risk of disease or recurrence of disease is present.
Probiotic:	a live microbial feed supplement which survives the stomach passage and beneficially affects the host animal by improving e.g. the intestinal microbial balance.
Prophylaxis:	any means taken to prevent disease, including the short-term use of antimicrobials in animals which one knows, or has good reason to expect, will be exposed to bacterial infection.
Salmonella:	a genus of bacteria most commonly associated with diarrhoea and food poisoning and which can also cause disease in farm animals.
Stamping out	Defined by OIE as the slaughter of all infected and in-contact animals, together with cleaning and disinfection, and all the other measures that are necessary
Systemic treatment:	drugs by injection or absorbed when given by mouth and distributed through the body via the bloodstream.
Therapeutic use:	antimicrobials administered to treat individual humans or animals (or groups of animals) suffering from a bacterial infection.
Zoonosis:	infection by micro-organisms that can be transmitted from animals to humans, for example, salmonellosis and rabies. To include the definition provided at the new legislation

ANNEX 1

Figure 3 Poultry meat line (GGP: Great grand parent; GP: Grand parent, P: parent, b: broiler)



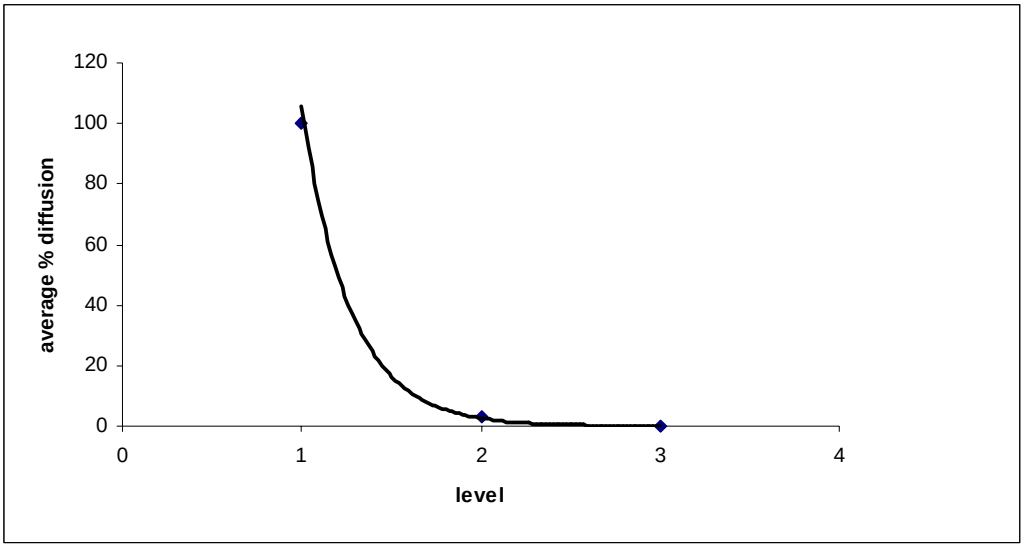
From 1 Great grandparent, 156.000-280.000 broilers are produced. If the probability of transmission from one level to the other is supposed to be 1, then one Great grand parent harbouring a resistant strain could theoretically lead to the contamination of 156.000-280.000 broilers (30*40*130; 40*50*140). One grandparent harbouring a resistant strain could theoretically lead to the contamination of 5200-7000 (40*130; 50*140) broilers. And one parent harbouring a resistant strain could theoretically lead to the contamination of 130-140 broilers.

Considering the following levels: Great Grand parent level (1), grand parent level (2), Parent level (3) and Broiler level (4), the following data are obtained:

level	max. n° progeny	min. n° progeny	average n° progeny	% diffusion max	% diffusion min	% diffusion average
1	280000	156000	218000	100	100	100
2	7000	5200	6100	2.5	3.33	2.91
3	140	130	135	0.05	0.083	0.066

Analysing the average percentage of diffusion, the levels are best described by an exponential function, as represented in figure 4.

Figure. 4 Average percent distribution



The exponential function that best describes these data using the mathematical method of least-squares is y=a*b^x, where a=4033.57 and b=0.025 (I=0.067). At level 3 the risk of diffusion is very close to the risk at level 4, at level 2 the risk is about 3x, at level one is about 100x.

ANNEX 2

Quinolones

The quinolones are a group of synthetic substances used both in human and veterinary medicine. Older quinolones are primarily active against Gram-negative bacteria while the fluoroquinolones have a broader spectrum of activity.

Both older quinolones, such as flumequine and oxolinic acid, and the more recently introduced fluoroquinolones such as enrofloxacin, sarafloxacin and difloxacin are authorised in different EU Member States for use in poultry (EMEA 1999). The specific quinolones authorised or marketed, as well as indications for use, differ between member states. The most common indication for use both in broilers and turkeys is probably treatment or prevention of infections caused by *Escherichia coli* (septicaemia or air sacculitis). Fluoroquinolones may also be used in some countries prior to hatch to control vertical transmission of *Mycoplasma* spp (Prescott *et a.l*, 2000). In human medicine, fluoroquinolones are used for many indications, both in community care for e.g. cystitis, and in hospitals for, e.g. treatment of life threatening infections with Gram-negative bacteria such as bloodstream infections with *Salmonella enterica*.

The fluoroquinolones kill bacteria through binding to two enzymes involved in supercoiling and relaxation of bacterial DNA (topoisomerase II and IV) (SCVPH, 2003a). The (hitherto) most important mechanism of resistance to quinolones is alteration of the target structure though mutations in the genes encoding the enzymes. Other mechanisms of resistance are activation of efflux pumps and decreased permeability (SCVPH, 2003a).

Almost exclusively, *Salmonella* strains with resistance to fluoroquinolone antimicrobials such as ciprofloxacin (human medicine) and enrofloxacin (food animals) also exhibit high level cross-resistance to the older quinolones as typified by nalidixic acid. In contrast strains with resistance to nalidixic acid do not exhibit clinical resistance to fluoroquinolone antibiotics such as ciprofloxacin. However in such strains the MIC to ciprofloxacin is increased from less than 0.1µg/ml to about 0.5–1.0 µg/ml. Although this level is not regarded as clinically-significant there have been several reports of treatment failures at this level, leading to requests for a reevaluation of clinical breakpoints (see below).

Aminoglycosides

The aminoglycosides are a group of antimicrobials used both in human and veterinary medicine. Their activity is broad against Gram-positive and Gram-negative aerobic bacteria, but streptococci and enterococci exhibit a low susceptibility.

The specific aminoglycosides authorised for use in poultry in differ between the Member States, with spectinomycin, neomycin and apramycin being the most common (EMEA 1999). Another aminoglycoside, gentamicin, is authorised for use in other animal species. A common indication for use of aminoglycosides in poultry is probably prevention of infections caused by *E. coli*. In some countries, egg dipping in gentamicin is used to control infections with *Mycoplasma* spp (Prescott *et al*, 2000). In people, the usefulness of aminoglycosides is limited by their toxicity and by the fact that they are poorly absorbed from the intestine. Still, gentamicin, amikacin and netilmicin are important drugs used in hospitals for treatment of serious infections caused by Gram-negative bacteria including *Pseudomonas aeruginosa* Other indications are staphylococcal infections and, in combination with ampicillin, enterococcal infections.

The aminoglycosides kill bacteria by binding to 30S subunit of the ribosome, thereby affecting the protein synthesis (Yao and Moellering, 1999). Resistance is mainly caused by enzymatic inactivation of the drug. There are three classes of aminoglycoside modifying enzymes; acetyltransferases, adenyltransferases and phosphotransferases. Each of these classes has numerous members, and most of these enzymes have a specific substrate spectrum ranging from narrow to broad. The patterns of cross-resistance conveyed by the genes encoding these enzymes are therefore complex. The genes are generally transferable, being located on transposons, integrons and/or on plasmids. Other less important mechanisms of resistance are decreased drug uptake, efflux systems and, for streptomycin, mutations (Quintiliani *et al*, 1999).

Ceftiofur

Ceftiofur is an antibiotic belonging to the class of cephalosporins which are not currently authorised for use in poultry, though they may be used by 'off-label' prescription. Many cephalosporins of different generations are used in human medicine. Currently, only ceftiofur is widely used for systemic treatment of food animals. The spectrum of activity of different cephalosporins varies. Ceftiufur has a good activity against many Gram-negative and Gram-positive bacteria and its spectrum of activity is closest to that of the expanded spectrum cephalosporins (3rd generation), to which also drugs such as ceftriaxone and cefotaxime belong (Prescott and others, 2000). Ceftriaxone is used for treatment of poultry in some far eastern countries.

Ceftiofur is poorly adsorbed after oral administration, and any use is therefore limited to injections. The drug is authorised in some countries outside the EU for injection of newly hatched chickens or turkeys for prevention of *E. coli* septicaemia and other infections causing mortality in very young birds. Cephalosporins with a similar spectrum are widely used in medical settings (hospitals) for treatment of serious infections, including blood stream infections with *Salmonella* spp.

The mechanism of action of cephalosporins is the same as for penicillins. They interfere with the formation of the bacterial cell wall by binding to enzymes that are active in the synthesis of peptidoglycans (transpeptidases, also called penicillin binding proteins or PBPs). In staphylococci, resistance to cephalosporins is caused by alteration of the PBPs (methicillin resistant Staphylococci, MRS) (Yao and Moellering, 1999). Resistance to cephalosporins in Gram-negative bacteria is mostly caused by production of beta-lactamases with substrate specificity for cephalosporins, so called extended spectrum beta-lactamases (ESBL of Class A) or beta-lactamases of class C. Many enteric bacterial species, including *E. coli*, inherently carry *amp*C, a gene coding for a beta-lactamase of the CMY-2 type, with activity against cephalosporins such as ceftiofur. Normally, the production of the enzyme is repressed and the quantities insufficient to inactivate the drug. Various mutational events may lead to overproduction of the enzyme, with clinical resistance as consequence (Quintiliani, Sham and Courvalin, 1999). *Salmonella enterica* does not inherently carry the *amp*C gene, but acquired resistance due to a transferrable *amp*C gene located on different plasmids seems to be increasing in prevalence (Anderson and others, 2004). Transferable *amp*C mediated resistance has also been reported in *E. coli* from various animal sources, including poultry (Brinas and others, 2003).

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