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

(Question N° EFSA-Q-2003-080)

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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 vaccines against *Salmonella* spp. is an example of such potential specific control methods.

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. Vaccination of chickens is regarded as an additional measure to increase the resistance of birds against *Salmonella* exposure and decrease the shedding. Whether vaccination is a suitable option in a control programme or not, depends on the aim of control programme (reduction or eradication), type of poultry, stage of production, true prevalence of *Salmonella*, serovars targeted, detection methods used and cost-benefit.

The *Salmonella* vaccines currently authorized for use in poultry in the Member states have been authorised on the basis of the mutual recognition procedure. Both live and inactivated *Salmonella* vaccines are available. At the moment, the extent of vaccination of breeders and laying hens in different Member States differs considerably. The application of vaccines is recommended in some Member States and forbidden in others. Currently, broilers are only very seldom and turkeys not at all vaccinated in Europe.

The vaccines are targeted for the most often reported serovars of human infections in Europe (*S*. Enteritidis and *S*. Typhimurium). However, vaccination is not, at the moment, a control option for many other serovars which can be present on poultry farms. Infection of poultry by serovars other than *S*. Gallinarum and *S*. Pullorum does not generally induce clinical signs except in young birds. Apart from these cases, vaccination has a limited effect on improving animal health and welfare and is used primarily for public health reasons (*S*. Typhimurium and *S*. Enteritidis).

Vaccines can decrease public health risk caused by *Salmonella* in poultry products by reducing the colonisation of reproductive tissues as well as reducing faecal shedding. There is experimental and some limited field evidence that a reduced level of faecal excretion and systemic invasion of *Salmonella* organisms in vaccinated birds will result in a reduced contamination of table eggs and the environment. However, further

information is still needed on the level and on the duration of protection after vaccination under field condition

When vaccination is used in *Salmonella* control programmes, possible interferences with standard *Salmonella* bacteriological and serological detection methods may be a disadvantage. In addition, there is concern over the use of antimicrobial resistance markers in some vaccines. One possible disadvantage of the use of live vaccines would be the spread of the strain to environment or to humans. Experience based on widespread use of existing *Salmonella* vaccines over several years and the results of monitoring, indicates that the vaccine strains of concern have not been disseminated in the environment or to humans. Use of inactivated vaccines against *S.* Enteritidis may also interfere with surveillance and control programmes for *S.* Pullorum/Gallinarum.

The panel concludes that if a control programme is targeting for serovars *S.* Enteritidis and *S.* Typhimurium in breeders of layers/broilers or laying hens and the flock prevalence is high, vaccination may be useful in reducing shedding and egg contamination. If the flock prevalence is low, vaccination may not be so useful but still could be used as one of the preventive measures to maintain a low prevalence. Provided that the detection methods are able to differentiate the vaccine strain from wild strains, both inactivated and live vaccines can be safely used throughout the life of the birds except during the withdrawal period before slaughter. This applies to parent flocks of layers and broilers; it can also apply to grand parents flocks of layers and broilers.

In order to reduce shedding by pullets, live and/or inactivated vaccines can be safely used. In order to reduce shedding and egg contamination by layers, only inactivated vaccines can be used due to the risk of spreading vaccine strain to eggs. Since vaccination cannot guarantee freedom of *Salmonella*, and the consequences of spreading from the top of the pyramid of poultry production would be severe, it is unlikely to be considered in great grand parents of layers and broilers.

If a control programme is targeting to eradicate the serovars *S.* Enteritidis and *S.* Typhimurium in breeders of layers/broilers or laying hens, vaccination is not an option since it does not eliminate the shedding. Furthermore, if a control programme is targeting serovars other than *S.* Enteritidis and *S.* Typhimurium in breeders, layers, broilers or turkeys, vaccination is not an appropriate option since the other serotypes are not covered by commercial vaccines available at the moment.

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BACKGROUND

Salmonella spp. 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.

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. 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 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[3] is at the final stage of revision. It will soon be repealed and replaced by a Directive on the monitoring of zoonoses and zoonotic agents and a Regulation on the control of *Salmonella* and other specified zoonotic agents. 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 serovars 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 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 vaccines against *Salmonella spp.* is an example of such potential specific control methods. Such vaccines have been designed in the early 90s', using defined *Salmonella* serovars, such as *Salmonella* Enteritidis and *Salmonella* Typhimurium. Inactivated as well as attenuated vaccines are available. Besides their efficacy,

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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)

aspects such as safety and compatibility with programmes to monitor and control *Salmonella* need to be considered in a global manner, in order to put in place appropriate management measures, where necessary.

The issue of vaccines was touched upon in the Community scientific reports of 11 November 1993 and of 20 February 1995 (see below).

TERMS OF REFERENCE

The European Food Safety Authority is asked to

(1) identify the different types of available vaccines against *Salmonella* in poultry.

(2) indicate the scientific and practical advantages and disadvantages of the identified vaccines, in particular against their possible use in the framework of control programmes and taking into account the different types of flocks, such as *Gallus gallus* and turkey flocks as well as breeding, laying hen and broiler flocks.

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

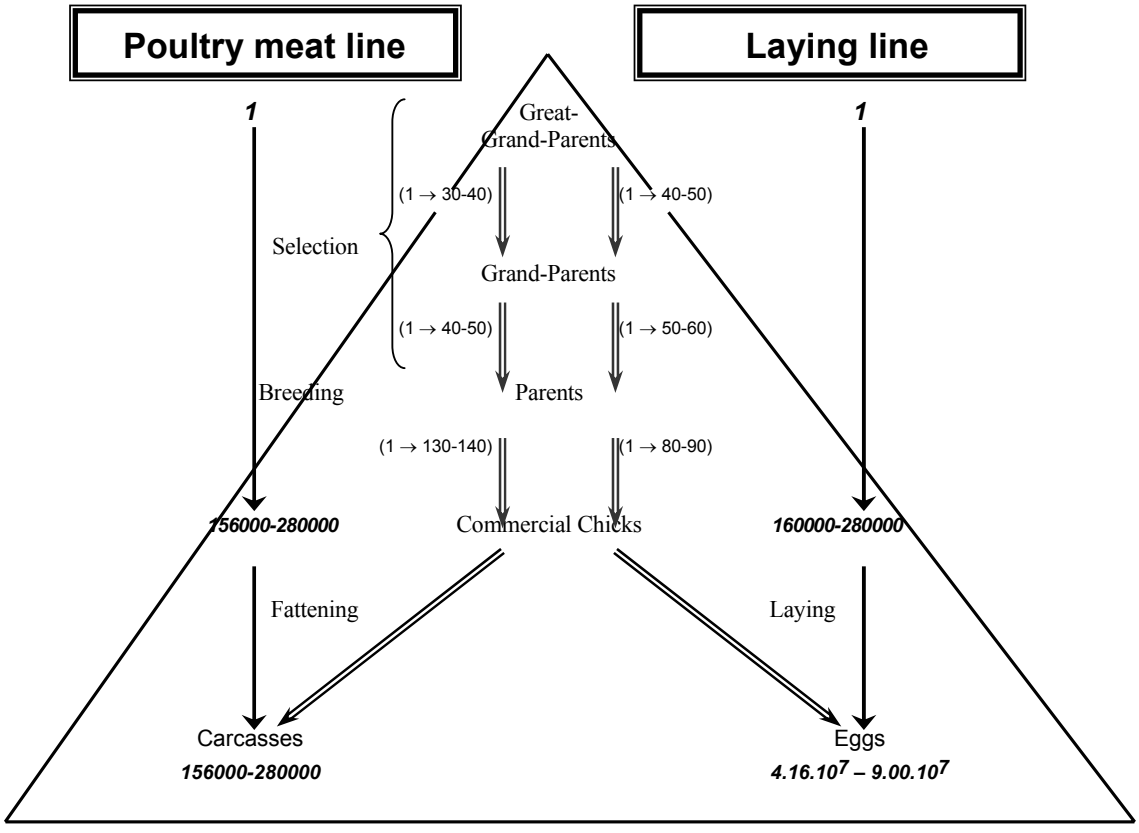

Figure 1. Simplified structure of poultry production

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/EC4, "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 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.

[4] 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.

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 (EFSA, 2004).

http://www.efsa.eu.int/science/biohaz/biohaz_opinions/723_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.*, 2002a). 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. VACCINES AVAILABLE FOR POULTRY

In *Salmonella* control in poultry production, the primary aim is to prevent the non-typhoidal serovars of *Salmonella* from entering the food chain via eggs or meat. The successful control of *Salmonella* in poultry is fundamentally based on the use of good hygiene and husbandry practice from stable to table (WHO Workshop, 1994). Successful control of *Salmonella* infections in poultry starts at the farm and includes qualified management in connection with strictly observed zoosanitary measures (e.g. litter, feed, water, rodents and birds). Additional effective specific control methods, e.g. vaccination and the use of competitive exclusion may also be used.

There has been an increasing interest to use *Salmonella* vaccination in poultry especially against the serovars of major public health relevance, *S.* Enteritidis and *S.* Typhimurium. Vaccination of birds results in a further increase of resistance against *Salmonella* infection beyond the level of birds

with developed intestinal flora. Inactivated and/or live *Salmonella* vaccines are in use for poultry in some countries.

The aim of vaccination as part of a complex control system for *Salmonella* infections in poultry is the prevention or reduction of intestinal colonisation resulting in reduced faecal shedding and egg shell contamination and also in reduced colonisation of reproductive tissues.

2.1. Scientific bases for vaccination

Immune protection of food producing animals including protection against re-infection with *Salmonella* serovars that induce systemic typhoid-like infections is strong and may be induced by live, attenuated vaccines. However, it is more difficult to demonstrate effective immune protection against intestinal infections caused by the vast majority of *Salmonella* serovars that do not produce systemic disease but colonise the gut efficiently.

Systemic infection or colonisation of the gut is not clear-cut. Research on murine typhoid has led to many discoveries in the field of *Salmonella* immunity. It is clear that extrapolation between different host species must be done with care, not only because of differences in the immune systems of different animals but also because of the difference in the nature of the diseases (Barrow, 1996).

In addition, some serovars cause systemic disease either in very young animals or in animals that are immunologically compromised, for example during pregnancy or as a result of concomitant viral or parasitic infections. Thus, *S.* Pullorum and *S.* Enteritidis may become associated with the reproductive tract in poultry at sexual maturity and although the basis for the latter is largely unknown it may present some difficulties in immune protection.

Thus, for poultry, **the aim of vaccination** is two fold:

- to prevent systemic infection and localisation in the reproductive tract
- to reduce faecal shedding and the carcass and/or egg contamination that results from it.

The basis for protection of poultry against *Salmonella* infection is largely empirical, although knowledge relating to the course of the innate and adaptive response to various *Salmonella* infections types is beginning to increase.

The nature of the early response and interactions with avian tissue is poorly understood and much of current knowledge is derived directly or indirectly

from studies on mouse typhoid. Interactions with components of the innate immune system, such as enterocytes, dendritic cells, neutrophils, the Toll-like receptors (TLR) and defensins and chemokines that they express which may define the nature of the adaptive response are being studied. It may also have a direct effect on the course of the infection. Thus, whereas *S.* Enteritidis, which is cleared from the intestine by an immune response and does not normally cause a systemic disease, stimulates high-level production of IL-1 and IL-6 from epithelial cells, *S.* Gallinarum and *S.* Pullorum suppress their production by these cells. Such differences may be responsible for the differing responses against *S.* Enteritidis, for example, and *S.* Pullorum, where a predominantly Th1-type response against Enteritidis may result in immune clearance whereas a predominantly Th2-type response against Pullorum may lead to prolonged infection and the carrier state. Vaccines are required to induce a Th1-type response that leads to immunity and clearance after challenge. Candidate vaccines that do not do fulfil this requirement will not reach the stage of field testing. The nature of the early response and the direction of the adaptive response may thus be crucial in determining whether a vaccine will be effective or not. The age of the bird will also be important in determining the nature of the immune response both because of the differential activity of components of the innate response, such as heterophils, but also by virtue of the differences in T cell populations after infection.

From murine and avian studies the dogma has arisen that live vaccines are more effective than killed vaccines, largely, because they stimulate both the cellular and humoral arms of the immune system but also because the nature of the response generated is a Th1 rather than a Th2 –type response, and it is regarded as important that this occurs for immune clearance. It is believed that both CD4+ and CD8+ T cells are required for immune clearance (Berndt and Methner, 2001) and despite the fact that on theoretical grounds, clearance from the intestine should involve primarily a humoral response there is mixed evidence that this is the major factor (Brownwell *et al*., 1970; Corrier *et al*., 1991; Desmidt *et al*., 1998b).

By contrast, a number of studies in chickens have been carried out in which approaches to vaccination and its limitations have been determined empirically but effectively. These have indeed shown that live vaccines are generally more effective against both intestinal and systemic infection than are killed vaccines. They also demonstrate varying degrees of protection induced by all of the killed and live vaccines that are currently available as commercial products. It is true that most currently available vaccines have been produced largely without recourse to our current understanding of host-pathogen interactions or avian immunology and do not use molecular biotechnology. The above is an indication that our understanding of the mechanism of immune protection against *Salmonella* infection in the chicken is rudimentary and insufficient to adopt a truly rational approach to

vaccine development and immune manipulation. There is also no information on the important bacterial immunogens. Their identification should be a significant research goal.

2.2. Serovars in vaccination and cross-protection between different serovars

As *S.* Typhimurium and *S.* Enteritidis are the serovars of *Salmonella* most important for public health in Europe, all existing commercially available *Salmonella* live and inactivated vaccines are intended for use against these serovars. Also the indication of a commercial live *S.* Gallinarum vaccine strain is the active immunisation of layers against *S.* Enteritidis. However, for other serovars relevant to human infections no vaccines are available for poultry production.

The salmonellae primarily responsible for enteritis in humans belong to a number of serogroups, including groups B, C and D. There is little evidence for any significant cross-protection between serogroups in mice (Lindberg *et al*., 1993; Segall and Lindberg, 1993; Hormaeche *et al*., 1996), cattle (Meyer *et al*., 1993; Villarreal *et al*., 1997) or chickens (Springer and Selbitz, 1996), Curtiss and Hassan, 1996) although the reason for this remains unclear. Some experimental evidence exists indicating little mutual protection between groups B and D in chickens. No published information exists for group C. There is some evidence, from western blots, that much of the response is to antigens that are common between different serogroups and that much of the response is therefore irrelevant (Hormaeche *et al*., 1990). It seems likely, therefore, that lipopolysaccharide (O-antigen) is a major component of the key immunogenic complement, although by no means the only one, and that protection between strains within a serovar is likely to be much greater. This assumption is supported by investigations in poultry under both experimental (Springer and Selbitz, 1996; Parker *et al*., 2001) and field conditions. After introduction of large scale vaccination using live *S.* Typhimurium in poultry breeding farms (layers and broilers) the detection rate of both *S.* Typhimurium and Enteritidis dropped considerably. Twelve months after starting vaccination, *S.* Typhimurium was no longer detected, indicating the strong homologous immunisation effect. The detection of *S.* Enteritidis was considerably reduced suggesting a partial cross immunity effect between serogroups B and D. However, as homologous immunity between strains of the same serovar is considerably stronger than between strains of different serovars, live *S.* Enteritidis vaccines were developed.

2.3. Types of vaccines available for poultry

Both live and inactivated *Salmonella* vaccines are available for poultry (Table 4 in Annex) and a variety of vaccine preparations has been developed and tested for their protective efficacy in poultry (Barrow *et al*., 1990; Barrow *et al*., 1991; Nagaraja *et al*., 1988; Cooper *et al*., 1992; Vielitz *et al*., 1992; Gast *et al*., 1993; Hassan and Curtiss, 1994; Cooper *et al*., 1994;

Nakamura *et al*., 1994; Curtiss and Hassan, 1996; Hahn, 2000; Springer *et al*., 2000; Feberwee *et al*., 2001).

Although a number of different **live** *Salmonella* strains have been tested for their efficacy in experimental or semi-field studies, only a few are authorized and commercially available for use in poultry in Europe. The accessible live *S.* Typhimurium and *S.* Enteritidis vaccine strains are either auxotrophic double-marker mutants derived through chemical mutagenesis (Meyer *et al*., 1993; Springer *et al*., 2000) or developed on the basis of the principle of metabolic drift mutations (Vielitz *et al*., 1992; Linde *et al*., 1997; Hahn, 2000). These are negative mutations in essential enzymes and metabolic control centres of the bacterium as a consequence of which the resulting metabolic process lead to prolonged generation times and corresponding reductions in virulence (Linde *et al*., 1993). Some of these *S.* live vaccines were further characterised by molecular methods (Schwarz and Liebisch, 1994). Another live vaccine authorized for prophylactic use against *S.* Enteritidis is based on a rough strain of *S.* Gallinarum without further molecular characterisation (Williams, 1956; Feberwee, *et al*., 2001).

Also a number of different **inactivated** preparations of *Salmonella* organisms have been tested for their efficacy against *Salmonella* challenge in poultry. However only one commercial inactivated *S.* Enteritidis based vaccine against *S.* Enteritidis infection in breeders and laying type chickens (Feberwee *et al*., 2000) is used in different countries and one commercial inactivated bivalent *S.* Enteritidis and Typhimurium dual vaccine against both *S.* Enteritidis and Typhimurium has been authorized recently (Clifton-Hadley *et al*., 2002). These killed vaccine types are based on bacterial cells cultured under conditions of iron depletion, despite the belief that iron does not to occur in the *Salmonella* containing vacuole in the macrophage (Eriksson *et al*., 2003). A more rational approach might be to culture cells under the conditions found inside the macrophage, as currently understood.

In some countries vaccination schemes using a combination of live and inactivated *Salmonella* vaccines are applied (Hafez *et al*., 2001). Usually live vaccines are administered orally via drinking water in very young chicks during the rearing period followed by parenteral injection of inactivated vaccines before the beginning and during the laying period. However, immunisation schemes that do not use combination of live and inactivated vaccines are also used (Feberwee *et al*, 2001; Cogan and Humphrey, 2003).

Commercially available live and inactivated *Salmonella* vaccines for use in poultry in Europe and other parts in the world are listed in Table 3 of the Annex. The information in the table was provided by the vaccine companies, and published in the Animal Pharmaceutical Report by Hales and Hales (2003). However, the list of products in Table 3 does not claim to be complete.

Although most of the *Salmonella* vaccines used in Europe are available commercially, also **autologous** vaccines can be used in some countries. An autologous vaccine is made by isolating a local strain of *Salmonella* spp. from a poultry house, either from the same group of animals or close environment, and producing a specific vaccine for this poultry farm. These vaccines comprise extracts of a culture killed by various processes (heat, formalin, etc.) and various adjuvants. Their efficacy is very controversial and their therapeutic efficiency has never been proven. These types of vaccines can only be produced for the farm of concern and they therefore are not authorized in any reports of the EU.

2.4. New vaccines in the future

Currently available vaccines are either inactivated whole bacterial culture (bacterins) or attenuated live strains produced by chemical mutagenesis and in which the nature of the attenuating lesions is unknown. There is room for debate on the relative merits of defined against undefined vaccines and the issue of genetic modification in bacterial vaccines generally, however, a list of desirable characteristics can be drawn up which has relevance to any form of vaccine (Pritchard *et al.*, 1978, Barrow and Wallis, 2000). The desirable characteristics are:

- a high degree of protection against systemic and intestinal infection
- adequate attenuation for both animals and man, should the strains enter the human food chain.
- the strains should not affect growth of the animal
- a degree of protection against a variety of important serovars (serogroups)
- vaccine strains should not be antibiotic resistant
- vaccines should be easy to administer and have markers facilitating tracing and identification.

There may be a requirement for a means of differentiation between field strains and vaccine strains and this has a bearing on compatibility between vaccine use and other means of control, whether this is based on bacteriological or serological monitoring.

The closer that live vaccine strains are to the parent strain from which they were derived, the greater the protection will resemble that observed in convalescent animals after natural infection by the wild-type strain. This is a "gold-standard" against which vaccine strains can be compared but which itself may be improved upon. Attenuation to prevent systemic disease, enteritis and intestinal colonisation is now possible through targeted

deletions of a selection of appropriate genes, including those in pathogenicity islands, many of which have already been identified as intellectual property. It is also possible to introduce mutations, which will eliminate the production of antigen specific flagella or LPS which could cause confusion in serological monitoring. However, such mutations may also affect the induction of early innate and adaptive responses. In addition the use of such mutations will enable easy differentiation from wild-type strain using PCR. The issue of the use of genetic manipulation for the production of vaccines for use in food animals is a major issue for public debate and which may even determine the nature of the vaccines used. Suffice is to say that rationally attenuated deletion mutants as vaccines can be safe and will in no way facilitate the introduction of new genes into the target microorganism, the host's microflora or the host itself.

Inactivated vaccines are likely to continue to be based on bacterins, cultured in ways to mimic the conditions inside macrophages. However, further research on *in vivo* gene expression and the *in vivo* proteome may identify major immunogens which might be incorporated into subunit vaccines or used with bacterins. It is certainly true that none of the major immunogens identified from western blotting are correlated with protection and thus much of the immune response to infection is thought to be irrelevant to protection.

2.5. Authorisation of vaccines in EU/National level

The *Salmonella* vaccines currently authorized for use in the Member states have been authorised on the basis of the national or mutual recognition procedure and in other countries in the world on the basis of national procedures.

For authorisation of *Salmonella* vaccines as immunological veterinary medicinal products in the EU, they have to meet the requirements of national regulations (e.g. Animal Vaccine Act in Germany) in the Member states and other countries in the world, and international guidelines, especially the i) Directive 2001/82/EC[5] of the European Parliament, the council of 6 November 2001 on the Community code relating to veterinary medicinal products, the ii) European Pharmacopoeia and iii) diverse European Guidelines.

There are different procedures for marketing authorisation of veterinary medicinal products according to Directive 2001/82/EC. A pharmaceutical company wishing to market a veterinary medicinal product in more than one Member state must use either the centralised procedure for veterinary medicinal products or the mutual recognition procedure (MRP). The mutual

[5] Directive 2001/82/EC of the European Parliament and of the council of 6 November 2001 on the Community code relating to veterinary medicinal products.

recognition can be achieved by asking other Member State(s) to mutually recognise, within 90 days, the marketing authorisation granted by the reference Member State (Directive 2001/82/EC). Thus rapid access to a single market, with the necessary safeguards for the protection of public health, can be obtained using the principle of mutual recognition.

The objective of this Community procedure is to facilitate access to a single market by relying upon the principle of mutual recognition. Thus with the exception of new biotechnology veterinary medicinal products which are subject to the centralised Community authorisation procedure by the European Agency for the Evaluation of Medicinal Products (EMEA), a marketing authorisation in one Member State ought in principle to be recognised by the competent authorities of the other Member States, unless there are grounds for supposing that the authorisation of the veterinary medicinal product concerned may present a risk to human or animal health or the environment. Examples for new biotechnology products, which would be considered obligatory for the Centralised Procedure in accordance with Regulation (EC) 726/2004[6] are i) products intended for gene therapy, ii) vaccines from strains developed by means of recombinant DNA technology, including gene deletion and insertion, iii) any veterinary medicinal product for which a monoclonal antibody is used and, iv) cell therapy products. Therefore, the commercially available live *Salmonella* vaccines which were produced by chemical mutagenesis or metabolic drift mutation (Meyer *et al*., 1993; Linde *et al*., 1997) were authorized in a number of Member States on the basis of the mutual recognition procedure.

2.6. State of the art of vaccination in Member States

The main focus of *Salmonella* vaccination in Member States has been placed on breeders and laying hens. At the moment, the degree of vaccination of poultry differs considerably between countries. There are also clear differences between countries with respect to the registration of inactivated versus live vaccines (Table 3).

In a number of countries vaccination of breeder birds for egg and meat production and chickens for table egg production using both live and inactivated vaccines is part of complex control programmes against *Salmonella* infection in breeder and layer birds. However, there is a wide spectrum of attitudes amongst countries to the use of vaccination against *Salmonella* in poultry at all and also to the use of live or inactivated vaccines. The application of vaccination is e.g. recommended/obligatory in Germany and Belgium, allowed in Austria, France, Greece, Hungary, Italy,

[6] Title III of Regulation 72&72004 establishing the procedure for the authorisation of veterinary medicinal products shall apply from 20 November 2005. Until that date, Title III of Council Regulation (EEC) 2309/93 applies.

Netherlands, Poland, Portugal, Spain and the United Kingdom and not accepted in Denmark, Finland, Ireland, Norway, and Sweden. (Report on Zoonoses, 2001, information from industry).

In the breeder sector, some countries (Austria, Belgium, Germany, Hungary, Italy, Portugal, Spain and United Kingdom) use both live and inactivated *Salmonella* vaccines whereas other countries (France, Greece, Netherlands, Poland,) use only inactivated vaccine preparations. In layer birds live *Salmonella* vaccines (Austria, Belgium, Hungary, Netherlands, Poland, Portugal), inactivated vaccines (France) or both vaccine preparations (Germany, Greece, Italy, Spain, United Kingdom) are in use**.**

3. ADVANTAGES AND DISADVANTAGES OF THE USE OF VACCINES

The development of vaccines to prevent poultry *Salmonella* infections has been a major challenge in the field of their control. An ideal *Salmonella* vaccine is one that is safe for poultry and other species, effective in eliminating the shedding of wild-type salmonellae and enhancing the clearance of these organisms from the host. Furthermore, the application of vaccines should not interfere with *Salmonella* detection methods.

Cost is a major consideration for any intervention; particularly for infections which themselves may not pose an economic problem to the poultry industry. Safety issues are also particularly important for pharmaceutical products (such as vaccines) which are used to protect against food borne pathogens and which may themselves pose a risk of entry into the food chain thereby infecting man. Furthermore, safety is dependent on quality and therefore the vaccine has to be thoroughly assessed. Contaminations in a vaccine can jeopardize national surveillance programs, diseases can spread and cause a danger to animals, human or the environment.

Safety for poultry, other animal species, humans and the environment as well as animal welfare issues are important when advantages and disadvantages of the use of vaccines are evaluated. On the other hand, the efficacy is also important.

3.1. Efficacy

The aim of vaccination in poultry is both the prevention and reduction of intestinal colonisation resulting in reduced faecal shedding and egg shell contamination and also in the reduced colonisation of reproductive tissues. Therefore, these criteria are generally included in potency testing of vaccines using quantitative and qualitative microbiological examinations of caecal content, cloacal swabs and different internal organs Usually, the basis for testing the efficacy of any vaccine preparation are experimental studies. Results from these experiments may be supplemented by field- or semi-field studies.

In the UK, there is strong circumstantial evidence that the introduction of vaccination in the broiler breeder sector, combined with improved hygiene and biosecurity, was fundamental in breaking the cycle of persistent farm

infection, hatchery contamination and dissemination of *S.* Enteritidis infection (ACMSF, 2001).

3.1.1. Experimental studies

Efficacy of vaccine preparations as judged by the level of intestinal and systemic colonisation and morbidity and mortality rates after vaccination and experimental infection using the oral or parenteral route of administration are examined. It is generally accepted that live *Salmonella* vaccines are more effective against both intestinal and systemic infection than are inactivated vaccine preparations (Lillehoj *et al*., 2000). However the level of protection depends on the challenge strain, the route of administration, the infection dose, parameters used to evaluate the course of infection, age of birds and species of birds. Consequently it is not possible to compare the efficacy of the vaccine preparations available.

One important reason for the difficulties in comparing the protection level is also the fact that an infection of chickens by the natural route even with an non-attenuated *Salmonella* wild-type strain will not result in absolute protection, e.g. another exposure of these birds with *Salmonella* organisms might result in a short-term intestinal colonisation. However, it was clearly demonstrated that vaccination of chickens results in a considerable quantitative reduced level and duration of intestinal colonisation and a diminished systemic invasion by *Salmonella* challenge organisms. (Barrow *et al*., 1991; Cooper *et al*., 1992, 1993; Curtiss and Hassan, 1996; Hahn, 2000; Springer *et al*., 2000; Feberwee *et al*., 2001, Clifton-Hadley *et al*., 2002). Reduction of egg contamination following vaccination has also been shown with some vaccines under experimental conditions (Woodward et al., 2002). Compared to the use of *Salmonella* wild-type strains as vaccines a number of registered *Salmonella* live vaccines revealed a high protective level (Hahn, 2000; Springer *et al*., 2000; Methner *et al*., 1995b, 2001)

In addition to the induction of a strong adaptive immune response, oral administration of live *Salmonella* bacteria to young birds results in extensive intestinal colonisation which confers additional protective effects which are potentially of great value. These include a colonisation-inhibition, similar to the competitive exclusion effect produced by gut flora preparations (Barrow *et al*., 1987b). This can prevent colonisation by wild-type *Salmonella* strains; however, current vaccine strains contain mutations which abolish this effect, suggesting that currently available vaccines may be effective as immunogens but will not be effective at competitive exclusion. Beside the microbiological colonisation-inhibition effect, the presence in the intestine of large numbers of live *Salmonella*-derived vaccine bacteria early post-hatch can induce infiltration of granulocytes into the intestinal mucosa and sub-mucosa, which confers resistance to invasion and systemic spread by virulent *Salmonella*

strains and, in mammals, gastro-enteritis (Van Immerseel *et al*., 2002b). This opens new perspectives for vaccine usage in broilers, layers and breeders.

Parenteral admínistration of inactivated *Salmonella* vaccines to breeder birds will induce a strong production of antibodies. These antibodies will be transferred to the progeny. The maternally transferred antibodies persist a few weeks and, although there seems to be some protective effect against disease in the early post-hatch period, there is little effect on intestinal colonisation by challenge strains (Methner *et al*., 1994; Methner and Steinbach, 1997)

3.1.2. Field trials

Studies on immunisation against *Salmonella* under field conditions also demonstrated the reduction of contamination and faecal shedding by both live and inactivated *Salmonella* vaccines (Timms *et al*., 1994; Linde *et al*., 1997; Feberwee *et al*., 2000, 2001; Davies and Breslin, 2003, 2004). However, it is very difficult to prove reduction of egg contamination following vaccination under field conditions owing to the low and variable percentage of contaminated eggs laid and the missing of appropriated non-vaccinated control flocks (Davies and Breslin, 2004).

The highest degree of protection was found in the case of low *Salmonella* exposure to the flocks, in a highly *Salmonella* contaminated environment (poor cleaning and disinfection, insufficient control of rodents etc.) the level of protection in vaccinated flocks was diminished (Davies and Breslin, 2003).

The most important aim of vaccination against paratyphoid *Salmonella* serovars in poultry is both the prevention and reduction of intestinal colonisation resulting in reduced faecal shedding and egg shell contamination and also in the reduced colonisation of reproductive tissues.
The commercially available *Salmonella* vaccines, registered in one or more countries of the EU fulfil these criteria as demonstrated at least under experimental conditions. It can be assumed that vaccines producing these effects will result in both a lower number of primary (infection within a hen before lay) and secondary infected or contaminated (infection of egg-shell due to faecal contamination) table eggs. It could be shown that the number of shell contaminated eggs declines with the reduction of intestinal *Salmonella* colonisation and faecal excretion in layers (Gast and Beard, 1990; Methner *et al*., 1995a). Recent studies (Timms *et al*., 1994; Woodward *et al*., 2002; Davies and Breslin, 2004) could confirm these effects under field conditions.

In both the UK and Germany there is strong circumstantial evidence that the introduction of vaccination in the breeder and layer sector, combined with

improved hygiene and biosecurity, was fundamental in breaking the cycle of persistent farm infection, hatchery contamination and dissemination of *Salmonella* Enteritidis infection resulting in a decrease of table egg contamination (Hartung, 2002a, 2002b; FSA,2004).

Also reducing shedding by vaccination may help to reduce the contamination of the environment as shown after introduction of large scale immunisation using live *S.* Typhimurium in layer and broiler breeding farms where the detection rate of these serovars dropped considerably (Vielitz, 1993). Twelve months after starting vaccination *S.* Typhimurium was no longer detected.

It must be stated, however, that scientific data on the level of protection and on the duration of protective effects after vaccination under field conditions are still incomplete, although it is experimentally very difficult and very expensive to confirm an effect with an adequate number of unvaccinated control groups.

3.1.3. Birds for meat production

The requirements for immune protection of broilers are very different to those for layers. In broilers protection is required against a wide range of *Salmonella* serovars and over the relatively short period of life of the bird. It is therefore an advantage that oral administration of live vaccines confers a number of non-specific prophylactic effects against infection.

Salmonella vaccines are only rarely used for broiler flocks since the cost of treatment, lack of positive effect, need for many serovars (not only *S.* Enteritidis and *S.* Typhimurium) and the short life span of the birds. They have been used only very little for turkeys. Until now live and inactivated *Salmonella* vaccines have not been authorised for use in turkeys in EU. Therefore, it is at the moment impossible to evaluate the prophylactic and therapeutic effects of *Salmonella* vaccines available in those species.

3.2. Safety for poultry and other animal species

Studies on safety and the risks that could arise from the use of immunological veterinary medicinal products are an obligatory part of the authorisation procedure of the products and include studies on safety, dissemination of the product in the environment and potency testing.

The requirements for safety testing include: the safety test shall be carried out in the target species, safety of the administration of one dose by each recommended route of administration to animals of each species, the category in which it is intended for use, safety of an overdose, safety of repeated administration of one dose, examination of reproductive performance and examination of immunological functions.

Live (*Salmonella*) vaccines have to meet further requirements. As an important criterion for their use, they have to be investigated comprehensively for their virulence in the target and non target-species. In addition to avirulence for poultry, avirulence for man, in an appropriate infection model such as gnotobiotic pigs (Barrow *et al*., 2001) or ligated intestinal loops (Wallis and Galyov, 2000) is recommended. Furthermore, live vaccines need to be tested for their ability to spread from vaccinated to unvaccinated target and non-target animal species. Additionally, data on the dissemination of the vaccine in the immunised animals have to be delivered. Faeces, eggs and different secretions must particularly be tested for the presence of the vaccine organisms. For live vaccines against established zoonotic diseases in food producing animals, studies on the dissemination and persistence of the vaccine strain in the body, with particular attention to the predilection sites for replication of the organism are obligatory. Another important safety parameter for *Salmonella* live vaccines is the investigation of reversion to virulence of attenuated vaccines or the potential that antibiotic resistance genes can be transferred to other microorganisms. Information on the biological properties of the vaccine strains have to be given, the probability of recombination or genomic re-assortment with field or other strains should be considered.

Genetic stability should also be considered as an important aspect of the safety of live *Salmonella* vaccines (Barbezange et al, 2000a)

Studies on persistence on the carcass and in food, with the vaccine regarded as a residue, are recommended as laboratory studies have shown that some live vaccines can persist in birds for several weeks (Barbezange et al, 2000b). A withdrawal period will be made to avoid the possibility of neither any residue nor any live vaccine organisms entering the food chain. This is of particular relevance for vaccines which might be considered for oral administration. Results from laboratory studies will be supplemented with supportive data from field studies.

Comprehensive studies on safety, efficacy and the risks of end product or environmental contamination of *Samonella* live and inactivated vaccines are an essential part of the documents for authorisation and registration of the product.

3.3. Safety for humans

3.3.1. Vaccination of breeders and layers

Vaccination programmes should ideally ensure that end products (poultry, meat and eggs) are not contaminated with either live vaccine strains or residues from inactivated vaccines.

The administration of live *Salmonella* vaccines in breeder birds and in chickens for table egg production has to guarantee by determining the

appropriate withdrawal period, that *Salmonella* vaccine organisms do not enter the food chain via contaminated eggs (Directive 2001/82/EC). Therefore the use of live *Salmonella* vaccines in layer chickens is restricted to the rearing period. The period between the last application of the live vaccine strain and the beginning of the laying period must be long enough to ensure that no live *Salmonella* organism is excreted by the animals.

The use of inactivated *Salmonella* vaccines in chickens for egg production is desirable from the point of view of absence of live *Salmonella* vaccine organisms from the end product.

Since 1994 it has been obligatory in Germany to vaccinate all layer chickens during their rearing period using live *S.* Typhimurium or *S.* Enteritidis vaccines. Several hundred million doses of live vaccines from different producers have been administered since that time and there has been no evidence of reversion to virulence. The competent authorities have not received any single notification of the presence of live *Salmonella* vaccines in eggs.

Because of the broad use of live *Salmonella* vaccines in different animal species (poultry, calves, pigs, pigeons), in Germany the competent authority has established a lab-based surveillance system for the occurrence of these live vaccine strains in the human population (Rabsch *et al*., 2001). Since 2000 all strains of *S.* Typhimurium and Enteritidis from humans were examined in respect to their phage types and vaccine markers. From several thousands isolates tested no live vaccine organism could be detected from infections in humans.

3.3.2. Vaccination of birds for meat production

From the public health point of view, the use of inactivated *Salmonella* vaccines in flocks for poultry meat production is preferable due to the absence of live *Salmonella* vaccine organisms in the end product. However, inactivated *S.* Enteritidis and *S.* Typhimurium vaccines have not been used so far in broilers as beside these serovars, a number of other *Salmonella* serovars is detected in broilers with a different spectrum in different areas in one country and between different countries. The application of live *Salmonella* vaccines in broilers would be possible, however, their use has to be guaranteed by determining the appropriate withdrawal period that *Salmonella* vaccine organisms do not enter the food chain (Directive 2001/82/EC).This is important since some laboratory studies have shown that some live vaccines can persist in birds for several weeks (Barbezange et al, 2000b). The use of killed vaccines will ideally require two dose vaccinations. Currently *Salmonella* live and inactivated vaccines have not been authorised for use in turkeys.

If live vaccines were used in broilers, an appropriate withdrawal period must guarantee that the vaccine organisms do not enter the food chain.

3.4. Environmental contamination

As *S.* Typhimurium and *S.* Enteritidis represent serovars that are not adapted to a particular host, their spectrum of hosts include poultry, cattle, pigs, mice, and numerous other animal species, including humans. Therefore, attenuated live vaccines from these serovars will also share that host spectrum. Transmission of the vaccine strain to other animal species at the farm or wild animals that are present in the area in which vaccinated birds are kept cannot be ruled out completely. These risks are substantially reduced however by adherence to the principle of good animal husbandry and animal hygiene. Furthermore, as the vaccine strain only colonises the vaccinated birds for a short period, even at the high doses they are given, the accidental uptake of small doses of the vaccine strain will not lead to any long-term colonisation of other animal species. In experimental and long-term field studies it has never been found that the authorized attenuated live *Salmonella* vaccines are able to develop chains of infection in primarily non-vaccinated animal species (Documents for authorisation of vaccines according to Directive 2001/82/EC) (Feberwee *et al*, 2001).

The use of inactivated *Salmonella* vaccines in animal species will not result in environmental contamination.

3.5. Gene exchange

Analysis of the *Salmonella* genome has identified a number of prophage elements and prophage component genes whose location in association with genes of differing GC values associated with t-RNA genes suggest that phages have been responsible for introduction of a number of virulence genes into *Salmonella enterica* (Boyd and Brussow, 2002). The *Salmonella* pathogenicity islands (SPI) differ in GC content to the rest of the genome suggesting an external origin. *S. bongori* does not possess a number of these virulence gene clusters. There is evidence for induction of phages with the transfer of the associated genes.

Transfer of virulence functions from virulent *Salmonella* to live vaccines produced by deletion mutation should result in strains which remained attenuated or would probably be no more virulent than the donor strain.

The currently available live vaccines have been produced largely empirically and indeed possess some characteristics, such as rifampicin resistance, which are not desirable. Antibiotic resistance of this sort can be transmitted by transduction via bacteriophages (Smith, 1972; Barrow *et al*., 1998). In the field of human medicine a more rational approach has been initiated through the production of mutants which are auxotrophic, such as those with mutations in the *aro* and other genes. *Aro* mutations, in particular, either as single or multiple mutations have been studied in depth. Such mutants are

unable to multiply intracellularly, and may be developed such that they are attenuated for poultry and man. Deletion mutants may also be developed which contain no extraneous DNA. Studies have been carried out in poultry indicating the value of some of these strains. However, there is current concern over the use of recombinant DNA technology in the construction of such strains. Progress in this area has ceased because of the fear of recombinant technology. Such issues require discussion in depth for rational resolution.

It is possible that vaccination may select for strains of *Salmonella* that avoid the immunological protection induced by vaccination. Changes in the genetic "make-up" of *Salmonella* result from horizontal transfer of genes or mutations of an organisms own DNA. Enterobacteriacae may use a variety of mechanisms, such as self-replicating plasmids, prophages, transposons, integrons etc (Normark and Normark 2002), to alter their DNA and which may aid the development of strains not covered by vaccination. It may be undesirable therefore, to base strategies to control *Salmonella* in poultry on vaccination alone**.**

3.6. Animal welfare

The adaptability of domestic poultry has been taken into account by breeders selecting breeding stocks. Breeding for high productivity can lead to decreased welfare but any selection that reduces the impact of pathogens will lead to improved welfare. Similarly, whilst some management practices can cause poor welfare, good management practices that avoid destructive behaviour, prevent disease, and promote good health will generally result in good welfare.

Among these practices, prevention of disease, or rapid diagnosis and treatment have a great impact on the welfare of chickens, laying hens and turkeys. Measures taken to prevent transmission of diseases among flocks and to control avian pathogens will help to optimise welfare. Thus, vaccination against common poultry diseases will improve the animal welfare provided that it is effective. Oral administration of vaccines can be used with welfare advantages: administration in drinking water is preferable than parenteral administration by injection as handling poultry may be stressful and the injection itself can be painful.

Concerning the possible impact of vaccination against non-typhoid avian salmonellosis on welfare, it is important to consider that infection by the *S.* Enteritidis, *S.* Typhimurium, etc. does not generally induce clinical signs except in young animals. It is rather characterised by silent carriage of bacteria with little impact on welfare but with a great impact for sanitary quality of poultry products e.g. eggs, meat; in these cases, vaccination has a more limited effect on improving animal welfare except in very young birds.

4. USE OF VACCINES IN CONTROL PROGRAMMES

There are some concerns that the use of vaccines in control programmes may interfere with:

a) detection methods
b) other control measures.

However, a number of these issues can be overcome as explained below.

Although it has clearly been demonstrated that vaccination of poultry results in a decreased shedding of either *S*. Enteritidis or *S*. Typhimurium, it should be imbedded in a general good hygienic practice programme. The benefits of using vaccination in a control programme depend on the:

- Aim of control programme (reduction or eradication)
- Type of poultry
- Stage of production
- True prevalence of *Salmonella*
- Detection methods
- Cost-benefit.

4.1. Possible interference between *Salmonella* detection methods and vaccination

The use of vaccination may have implications for detection of *Salmonella* in control programmes. Ideally, the vaccine strain itself or the antibodies produced by vaccinated birds should be easily differentiated from the wild *Salmonella* strains. Possible bacteriological and serological interference with standard *Salmonella* diagnosis when using vaccination have to be considered in control programmes.

Also, as the effect of vaccination is claimed to be a reduced excretion of *Salmonella* from infected flocks, this has to be taken into consideration, along with other successful control measures that more sensitive detection may be required for surveillance of vaccinated flocks, e.g. the number of samples may have to be increased in order to obtain a suitable detection limit for flocks with low prevalence infections.

4.1.1 Bacteriological detection methods

Live *Salmonella* vaccines have, according to the rules in the authorisation process, to be differentiable from *Salmonella* wild-type strains by bacteriological methods (Directive 2001/82/EC).

Currently, the live *Salmonella* vaccines can be differentiated from field strains on the basis of their defined auxotrophy, their resistance to defined levels of certain antimicrobials, or their defective LPS structure (Table 3).

Live vaccines excreted into the environment may mask the detection of wild-type *Salmonella* strains for a very short time after vaccination. Therefore, samples for the detection of wild-type *Salmonella* should be taken only some days after administration of the live vaccine.

Newer methods, such as PCR, may be in future used to easily differentiate vaccine strains from field strains, provided sufficient information is available on the genome of the vaccine strain.

Inactivated vaccines do not interfere with cultural aspects of detection of *Salmonella*.

4.1.2 Serological detection methods

The production of circulating antibodies after administration of live or inactivated vaccines can interfere with the results of serological investigation (Feld *et al*., 2000). It is therefore necessary to produce marker vaccines for both, live and inactivated vaccines, which produce a serological response that can be easily distinguished from an actual infection (Davies and Breslin, 2003).

A strategy of vaccination with a live vaccine strain based on *S.* Gallinarum (which has no flagellae) combined with a serological method based on flagellum proteins allowed a differentiation between infected and vaccinated flocks. However, the specificity may need some improvements (Feberwee *et al*., 2001, Solano *et al*., 2000). For other live vaccines this approach is not valid, and await the development of marker vaccines containing specific antigenic determinants allowing for a serological differentiation between antibodies produced after immunisation with live or inactivated vaccines or field infections, respectively..

However, most of the current inactivated vaccine preparations available which are administered parenterally will stimulate high titres of circulating *Salmonella* specific IgG which will strongly interfere with serological methods. Until now there are no serological test systems enabling the differentiation between titres induced by *Salmonella* wild-type infection and titres stimulated by inactivated vaccines.

The use of serological tests for surveillance of *Salmonella* in poultry is relatively unproblematic in control programmes without using vaccination and aim to ensure freedom of *Salmonella* by eradication, i.e. a test-and-removal policy. In this setting a positive serological test may be interpreted as infection. The most extensive use of serology for this purpose has been

and is an integrated part of the Danish *Salmonella* Control Plan. The test has been designed to catch broadly by a combination of antigens from *Salmonella* Enteritidis (O: 9,12) and S. Typhimurium (O: 1,4,5,12) in one ELISA test (Mix-ELISA) combined with another test based on *Salmonella* Infantis antigens (O: 6,7 – Infantis ELISA) as the Danish programme has a no-*Salmonella* strategy, i.e. all serovars are included in the control programme (Feld *et al*., 2000, Gradel *et al*., 2001, Skov *et al*., 2002).

In other countries targeting the two major serovars *Salmonella* Enteritidis and *Salmonella* Typhimurium in accordance with the requirements of the Zoonosis Directive 92/117/EEC, a specific serological test is available for *S.* Enteritidis but not for *S.* Typhimurium (Feberwee *et al*., 2001). This appears to be well suited for this purpose, but will not, due to its specificity, provide information about other emerging serovars in poultry production. Antibody response produced by *S.* Enteritidis inactivated vaccines cannot be distinguished from wild type infection.

Finally, it should be mentioned that vaccination by *S.* Enteritidis will interfere with surveillance and control programmes for *S.* Pullorum/Gallinarum as vaccinated birds will exhibit false positive reactions in the slide agglutination test applied for the detection here.

4.2. Possible inference between vaccination and other control measures

Since vaccination alone cannot guarantee the freedom of *Salmonella*, good hygienic practices and biosecurity measures on pre-harvest, harvest and post-harvest level are essential. These measures can prevent re-infection and cross-contamination of *Salmonella* to the vaccinated flock. These measures are especially important in breeding flocks since *Salmonella* infection at that stage of production may be transmitted to further stages and to a significant number of birds.

Although vaccination will not have an effect on the efficiency of good hygiene practices and biosecurity measures, there are some other control measures on the farm where the use of vaccination may cause some compatibility problems e.g. antimicrobial treatment, treatment with competitive exclusion culture and feed additives:

Administration of parenterally administered inactivated *Salmonella* vaccines will not interfere with a simultaneous application of antimicrobials. A previous or simultaneous treatment with antimicrobials, present in the drinking water system, in eggs or in chickens and efficient against the vaccine strain may affect the viability of the live vaccine and consequently the efficacy of the vaccination. For further information on the use of antimicrobials in *Salmonella* control programmes, see EFSA (2004) http://www.efsa.eu.int/science/biohaz/biohaz_opinions/723_en.html

- Administration of parenterally injected inactivated *Salmonella* vaccines will not interfere with a simultaneous administration of a competitive exclusion (CE) culture. To ensure the efficacy of live S*almonella* vaccines they must be applied either prior to or simultaneously with the CE culture. CE treatment before administration of the live vaccine will prevent the colonisation of the vaccine strain and therefore its efficacy (Methner *et al*., 1999, 2001).

- Numerous prebiotics, probiotics, and fatty acid formulations are commercially available for feed or drinking water supplementation to control *Salmonella* infections (Van Immerseel *et al*., 2002a). Those that block adhesion and those that have a direct anti-*Salmonella* activity may interfere with oral vaccination with live vaccines.

In addition to these specific control measures, some other problems may arise in using vaccination in connection to *Salmonella* control programmes:

- The infection and immune status of a flock before live or inactivated vaccination may decrease the effect of vaccination. This may be the case when, before vaccination, a flock is already infected with the wild strain of *Salmonella*. Also the disease status of a flock, especially the immunosuppressive diseases, and mycotoxins in feed stuffs may interfere the immunological response of vaccinated birds.

- From a public health perspective, live and inactivated vaccines are tools targeted for *S.* Enteritidis and *S.* Typhimurium, currently the most often reported serovars for human infections in Europe. In addition, there may be some cross-protection also for serovars belonging to serogroup B and D. However, many other serovars can be present on poultry farms and for those vaccination is not a suitable control option. Serovars other than *S.* Enteritidis and *S.* Typhimurium are of special importance in broiler and turkey production although they may also infect laying birds.

4.3. Vaccination at different stages of production line

When the flock prevalence is high, vaccination is useful to reduce the prevalence and excretion of *S.* Enteritidis and *S.* Typhimurium to low levels. If the flock prevalence is low, vaccination may not be so useful but could be used as a preventive measure to maintain a low prevalence. If eradication is the target of the control programme, vaccination should be replaced by testing and removal.

In order to reduce shedding and egg contamination, both inactivated and live vaccines can be used throughout the life of the birds except during the withdrawal period before slaughter. This period is defined for each

registered vaccine. This applies to parents of layers and parents of broilers, it can also apply to grand parents of layers and broilers.

Since vaccination cannot guarantee freedom of *Salmonella*, and the consequences of spreading from the top of the pyramid can be severe, it is unlikely to be considered in great grand parents of layers and broilers.

In order to reduce shedding by pullets, live and/or inactivated vaccines can be used. In order to reduce shedding and egg contamination by layers, only inactivated vaccines can be used due to the possible egg contamination by vaccine strain.

There are no registered vaccines available for broilers or turkeys at the moment in the EU. If a vaccine is judged desirable for broilers or turkeys, it should provide protection against many serotypes inducing a very rapid onset of protective immunity and rapid clearance well before slaughter.

5. CONCLUSIONS

- Based on the available statistics, eggs and poultry meat are a significant source of human salmonellosis in Europe. The most common serovars detected are *S.* Enteritidis and *S.* Typhimurium, although in poultry meat and meat products also many other serovars are involved. *S.* Enteritidis has been associated especially with eggs and egg products.

- 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. Vaccination of chickens is regarded as an additional measure to increase the resistance of birds against *Salmonella* exposure and decrease the shedding.

Types of vaccines available for poultry

- The *Salmonella* vaccines currently authorized for use in poultry in the Member states have been authorised on the basis of the mutual recognition procedure. Both live and inactivated *Salmonella* vaccines are available.

- At the moment, the extent of vaccination of breeders and laying hens in different Member States differs considerably. The application of vaccines is recommended in some Member States and forbidden in others.

- These vaccines are tools targeted for the most often reported serovars of human infections in Europe (S. Enteritidis and S. Typhimurium). However, vaccination is not, at the moment, a control option for many other serovars which can be present on poultry farms

- The vaccination programmes used may differ for breeders and layers. Currently, broilers are only very seldom and and turkeys not at all vaccinated in Europe. The choice of the vaccine depends on several factors (e,g way of administration, serovars involved, duration of effect needed etc).

Advantages and disadvantages of the use of vaccines

- Vaccines can decrease public health risk caused by *Salmonella* in poultry products by reducing the colonisation of reproductive tissues as well as reducing faecal shedding.

- There is experimental and some limited field evidence that a reduced level of faecal excretion and systemic invasion of *Salmonella* organisms in vaccinated birds will result in a reduced contamination of table eggs and the environment. However, further information is still needed on the level and on the duration of protection after vaccination under field condition.

- As a number of different experimental strategies have been taken to study the efficacy of the *Salmonella* vaccines available, it is difficult to compare the commercial vaccine preparations for their level of protection, the duration of protection or safety for humans or environment.

- Infection of poultry by serovars other than *S.* Gallinarum and S.Pullorum does not generally induce clinical signs except in young birds. Apart from these cases, vaccination has a limited effect on improving animal health and welfare and is used primarily for public health reasons (*S.* Typhimurium and *S.* Enteritidis).

- One possible disadvantage of the use of live vaccines would be the spread of the strain to environment or to humans. Experience based on widespread use of existing *Salmonella* vaccines over several years and the results of monitoring, indicates that the vaccine strains of concern have not been disseminated in the environment or to humans.

- When vaccination is used in *Salmonella* control programmes, possible interferences with standard *Salmonella* bacteriological and serological detection methods may be a disadvantage. In addition, there is concern over the use of antimicrobial resistance markers in some vaccines

- Much monitoring for *Salmonella* in poultry is largely based on bacteriological methods at the moment. Taking into account that for most of the live vaccines, methods are available to distinguish wild-type strains from vaccine strains, there is little incompatibility between *Salmonella* detection and vaccination with live vaccines. For inactivated vaccines, there is no incompatibility between the detection of *Salmonella* infections by bacteriological methods and vaccination.

- Until now there are no serological test systems enabling the differentiation between titres induced by *Salmonella* wild-type infection and titres stimulated by inactivated vaccines. However, for at least one commercial live vaccine against *Salmonella* Enteritidis a serological test is available which targets an antigen lacking in the vaccine strain but usually present in field strains.

- The vaccination of a flock already infected with a wild *Salmonella* strain may decrease the spread of a wild strain within a flock, but will not provide further protection against other strains.

- Until now, only very few *Salmonella* vaccines are authorized or used in broiler flocks and none in turkey flocks in Europe due to the cost of treatment, lack of positive effect, need for many serovars (not only *S*. Enteritidis and *S*. Typhimurium) and the short life span of the birds. Therefore, it is at the moment impossible to evaluate the prophylactic and therapeutic effects of *Salmonella* vaccines available in those species.

Vaccination and *Salmonella* control programmes

- Although vaccination alone cannot eliminate *Salmonella* from a flock, it may compliment other available measures in combating *Salmonella*. Whether vaccination is a suitable option in a control programme or not, depends on the aim of control programme (reduction or eradication), type of poultry, stage of production, true prevalence of *Salmonella*, serovars targeted, detection methods used and cost-benefit.

- At the moment, the extent of vaccination of breeders and laying hens in different Member States differs considerably. The application of vaccines is recommended in some Member States and forbidden in others.

- If a control programme is targeting for serovars *S*. Enteritidis and *S*. Typhimurium in breeders of layers/broilers or laying hens and the flock prevalence is high, vaccination may be useful in reducing shedding and egg contamination. If the flock prevalence is low, vaccination may not be so useful but still could be used as one of the preventive measures to maintain a low prevalence.

- Provided that the detection methods are able to differentiate the vaccine strain from wild strains, both inactivated and live vaccines can be safely used throughout the life of the birds except during the withdrawal period before slaughter. This period is defined for each registered vaccine. This applies to parent flocks of layers and broilers; it can also apply to grand parents flocks of layers and broilers.

- In order to reduce shedding by pullets, live and/or inactivated vaccines can be safely used. In order to reduce shedding and egg contamination by layers, only inactivated vaccines can be used due to the risk of spreading vaccine strain to eggs.

- Since vaccination cannot guarantee freedom of *Salmonella*, and the consequences of spreading from the top of the pyramid of poultry production would be severe, it is unlikely to be considered in great grand parents of layers and broilers.

- If a control programme is targeting to eradicate the serovars *S*. Enteritidis and *S*. Typhimurium in breeders of layers/broilers or laying hens, vaccination is not an option since it does not eliminate the shedding.

- If a control programme is targeting serovars other than *S*. Enteritidis and *S*. Typhimurium in breeders, layers, broilers or turkeys, vaccination is not an appropiate option since the other serotypes are not covered by commercial vaccines available at the moment.

- Use of inactivated vaccines against S. Enteritidis may interfere with surveillance and control programmes for S. Pullorum/Gallinarum as vaccinated birds will exhibit false positive reactions in the slide agglutination test applied for the detection of exposure to salmonellae.

- There are only few registered vaccines available for broilers and none for turkeys at the moment in the EU. If a vaccination is judged desirable for broilers or turkeys, the vaccine used should provide protection against many serotypes induce a very rapid onset of protective immunity and rapid clearance of vaccine strain well before slaughter.

6. RECOMMENDATIONS

- General methods other than the use of vaccines and antimicrobials to prevent *Salmonella* infections in poultry flocks, for example feed controls, biosecurity measures, etc, should be promoted.

- Criteria more specific than those requested for the marketing authorization for accepting or not a new vaccine against *Salmonella* in poultry in control programs should be defined (for example a quantitative assessment of the faecal shedding)

- Due to public health concerns, it is desirable that all countries move closer to the *Salmonella*-free poultry production. In most EU countries this is already the strategy and absence of salmonellae can be maintained for much of the time.

- There is an urgent need to stimulate research regarding the pathogenesis of *S.* Enteritidis infection that results in egg contamination in laying hens.

- Persistent infections and carrier birds are chickens that harbour the *Salmonella* bacteria and may continuously or discontinuously excrete the bacteria in the faeces. These birds obviously do not succeed in clearing the infection and seriously hamper the monitoring and control programmes. The mechanism of development of the carrier state and the roles of *Salmonella* – host interactions and *Salmonella* – gut flora interactions in the carrier state warrant further study.

- The above is an indication that our understanding of the mechanism of immune protection against *Salmonella* infection in the chicken is rudimentary and insufficient to adopt a truly rational approach to vaccine development and immune manipulation. There is also no information on the important bacterial immunogens and their identification should be a significant research goal.

- In order to fully capitalise on the advantages of vaccination while at the same time being able to closely monitor the expected *Salmonella* prevalence reduction in EU poultry flocks, it is imperative that vaccines with effective markers be developed in conjunction with cheap and effective test systems allowing for easy detection of wild-type *Salmonella* while at the same time differentiating these from vaccine strains or vaccine reactions.

- Further investigation should be carried out to exploit the colonisation-inhibition phenomenon between *Salmonella* organisms.

- Investigation on the development of marker vaccines enabling both the bacteriological and serological differentiation from wild-type strains should be encouraged (TR: redundancy with the 2 bullets above?).

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8. GLOSSARY

Antibiotic:	a substance, produced by or derived from a micro-organism, which inhibits the growth of or destroys other micro-organisms.
Antimicrobial:	a drug 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.

Table 3: Some commercially available live and inactivated paratyphoidal *Salmonella* vaccines for use in poultry. The list does not lay the claim to be complete. . (Part of the information was provided by EMEA). Further information can be found at: http://www.hevra.org/vmri_spc/

Name	**Company**	**Serovar**	**Live/ inactivated vaccine**	**Registered in**	**Species of poultry**	**Markers in live vaccines for bacterial differentiation from wild-type strains**
Salmonella vac T	Lohmann Animal Health Cuxhaven, Germany	*Salmonella* Typhimurium	live vaccine	Bulgaria, Ecuador, Germany, United Kingdom, Israel, Libanon, Austria, Philippines, Poland, Thailand, Hungary, Cyprus	chickens for egg production, breeder birds	resistant to nalidixic acid (20 µg/ml), resistant to rifampicin (100 µg/ml), sensitive to erythromycin (15 µg/ml), phage type DT 9
Salmonella vac E	Lohmann Animal Health Cuxhaven, Germany	*Salmonella* Enteritidis	live vaccine	Belgium, Bosnia, Bulgaria, Czech Republic, Germany, Greek, United Kingdom, Ireland, Israel, Jugoslavia, The	chickens for egg production, breeder birds	resistant to streptomycin (100 µg/ml), resistant to rifampicin (100 µg/ml), sensitive to erythromycin (15 µg/ml), phage type 4

				Netherlands, Austria, Portugal, South Africa, Thailand, Czech Republic, UAE, Hungary, Cyprus		
Zoosaloral H	Impfstoffwerk Dessau Tornau GmbH, Dessau, Germany	*Salmonella* Typhimurium	live vaccine	Germany, Hungary, Austria, China	chickens for egg production, breeder birds	auxotroph for purine and histidine, phage type DT 9, detection media delivered by the producer
Salmovac SE	Impfstoffwerk Dessau Tornau GmbH, Dessau, Germany	*Salmonella* Enteritidis	live vaccine	Germany, Hungary	chickens for egg production, breeder birds	auxotroph for purine and histidine, phage type PT 4, lack of the serovar specific plasmid; detection media delivered by the producer
Gallivac SE	Merial, France	*Salmonella*	live vaccine	Czech Republic, Germany, Great	chickens for egg	auxotroph for purine and histidine, phage type PT 4,

		Enteritidis		Britain, Greece, Austria, Belgium, Luxembourg, The Netherlands, Poland, Portugal, Ireland, Italy	production, breeder birds	detection media delivered by the producer
Poulvac ST	Fort Dodge, Animal health, USA	*Salmonella* Typhimurium	live vaccine	Czech Republic, USA, Slovakia	poultry	
Poulvac SE	Fort Dodge	*Salmonella* Enteritidis	inactivated vaccine	USA	poultry	
Poulvac SE-ND-IB	Fort Dodge	Combination of *Salmonella* Enteritidis, ND and IB	inactivated vaccine	USA	poultry	
Nobilis Salenvac	Intervet International, Netherlands	*Salmonella* Enteritidis	inactivated vaccine	Belgium, Bulgaria, Brasil, Chili, Czech Republic, Germany, Spain, France, United Kingdom, Hungary, Indonesia, Italy, Japan,	poultry	

				Lithuania, Luxembourg, Latvia, Netherlands, Peru, Philippines, Poland, Slovakia, Thailand, Turkey, Venezuela, South Africa		
Nobilis Salenvac T	Intervet International, Netherlands	*Salmonella* Enteritidis and *Salmonella* Typhimurium	inactivated vaccine	Austria, Belgium, Czech Republic, Estonia, Germany, Denmark, Spain, France, United Kingdom, Greece Hungary, Italy, Lithuania, Luxembourg, Netherlands, Poland, Portugal, Slovakia, Turkey,	poultry	
Nobilis SG 9R	Intervet International, Netherlands	*Salmonella* Gallinarum vaccine against *Salmonella* Enteritidis and	live vaccine	Argentina, Austria, Bangladesh, Belgium, Bulgaria, Bolivia, Brasil, Costa Rica, Czech Republic, Germany, Spain, France, United	poultry	antigenic response as *Salmonella* Gallinarum, defective in LPS, rough

		Salmonella Gallinarum		Kongdom, Greece, India, Italy, South Korea, Lithuania, Latvia, Morocco, Netherlands, Peru, Philippines, Pakistan, Poland, Portugal, Thailand, Uruguay, Vietnam, South Africa, Zimbbwe		
Megan ™ Vac 1	Megan Health, Inc., USA	*Salmonella* Typhimurium	live vaccine	USA, New Zealand	chickens	gene deletions in cya and crp,, biochemical response as *Hafnia alvei*, antigenic response as *Salmonella* Typhimurium
Gallimune SE	Merial, France	*Salmonella* Enteritidis	inactivated vaccine	Czech Republic, Poland, Slovaakia	chicken	

Immunovac	Biowet, Poland	*Salmonella* Enteritidis, Typhimurium, Seftenberg, Kentucky, Cholerasuis, Heidelberg, Gallinarum	Inactivated vaccine		chicken	http://www.biowet.pl/pl/badania/immunovac.htm
TAD Salmonella vac T	Lohman Animal Health GmbH&Co, Germany	*Salmonella* Typhimurium	Live vaccines	Germany (Reference Member State) Authorised through Mutual Recognition	Layers, brreders, broilers	Defective gyrase affecting DNA replication (nalidixic acid resistance) and defective RNA polymerase (Rif resistance).

Table 5. Advantages and disadvantages of the use of **live** and **inactivated** vaccines in *Salmonella* control programmes.

Objective	**Advantages**	**Disadvantages**
Public health	• Vaccines can decrease public health risk caused by *Salmonella* in poultry products by reducing the colonisation of reproductive tissues as well as reducing faecal shedding. This results in lower contamination level of eggs and poultry meat.	• Vaccines are tools targeted for the most often reported serovars of human infections in Europe (*S.* Enteritidis and *S.* Typhimurium). However, vaccination is not a suitable control option.for many other serovars which can be present on poultry farms Gene exchange between live vaccine strain and wild strains possible • Antimicrobial resistance markers of live vaccines have caused concern • Only very few scientific publications on the quantity of the decrease in secretion of *Salmonella* into faeces or on the contamination of eggs and environment in field conditions are available. • If withdrawal periods are not followed, live vaccine strains may contaminate the end product (eggs or meat).It is possible that vaccination may select for strains of Salmonella that are to avoid the immunological protection induced by vaccination.
Animal welfare		• Infection by the other serovars of *Salmonella* than Pullorum or Gallinarum, does not generally induce clinical signs except in young animals. In these cases, vaccination has a limited effect on improving animal health and welfare and is used mainly for public health reasons. • Parenteral administration by injection as well as handling poultry may be stressfull and injection itself can be painful.
Environment	• Vaccination may results in decreased environmental contamination of wild *Salmonella* strains. • In experimental and long-term field studies it has never been found that the authorised attenuated live vaccine would be able to develop chains of infection in primarily non-vaccinated animal	

Objective	Advantages	Disadvantages
	species. • The use of inactivated vaccine will not result in environmental contamination.	
Detection of *Salmonella* by bacteriological methods	• Live *Salmonella* vaccines have, according to the rules in the authorisation process, to be differentiable from *Salmonella* wild-type strains by bacteriological methods. • Currently, the live *Salmonella* vaccines can be differentiated from field strains on the basis of their defined auxotrophy, their resistance against a defined level of certain antimicrobials, or in defective LPS structure. • In general, the use of inactivated vaccines does not interfere with the bacteriological methods for *Salmonella* detection. However, live vaccines excreted into the environment may mask the detection of wild-type Salmonella strains for a very short time after vaccination. Therefore	• The detection of the infection of wild strain may not be possible after short period of vaccination
Detection of *Salmonella* by serological methods	• The use of serological tests for surveillance of *Salmonella* in poultry is relatively unproblematic in control programs aiming to ensure freedom of *Salmonella* by eradication, i.e. a test-and-removal policy.	• Oral administration of live *Salmonella* vaccines to chickens stimulates the production of lower antibody titres than those generated in a field infection. Therefore, by defining the threshold in an ELISA-systems it might be possible to differentiate flocks vaccinated. However currently such methods are not commercially available. • Until now there are no serological test systems enabling the differentiation between titres induced by *Salmonella* wild-type infection and titres stimulated by inactivated vaccines. • Vaccination for paratyphoid *Salmonella* like S. Enteritidis and S. Typhimurium may interfere with surveillance and control programmes for S. Pullorum/Gallinarum as vaccinated birds will exhibit false positive reactions in the slide agglutination test applied for the detection here.

Objective	Advantages	Disadvantages
Competitive exclusion	• Administration of parenterally injected inactivated *Salmonella* vaccines will not interfere with a simultaneous administration of a competitive exclusion (CE) culture. • To ensure the efficacy of live *Salmonella* vaccines they must be given either prior to or simultaneously with the CE culture.	• CE treatment before administration of the live vaccine will prevent the colonisation of the vaccine strain and therefore reduce its efficacy.
Pre- and probiotics	• There is no systematic investigation of the effect of prebiotics, probiotics, symbiotics and fatty acid formulations on the eradication of *Salmonella* spp. in poultry. • Prebiotics 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.	• Those that block adhesion and those that have a direct anti-*Salmonella* activity may interfere with oral vaccination with live vaccines.
Antimicrobial treatment	• Parenterally administered inactivated *Salmonella* vaccines will not interfere with a simultaneous application of antimicrobials.	• A previous or simultaneous treatment with antimicrobials, present in the drinking water system, in eggs or in chickens and efficient against the vaccine strain may affect the viability of the live vaccine and consequently the efficacy of the vaccination. • If coccidiostats with antimicrobial effect are used, there may also be some effect on the vaccination efficiency. However, for inactivated vaccination this risk is low.
Good farming and hygiene practices (GFP and GHP)	• Vaccination may decrease the excretion of *Salmonella* in faeces and therefore add in on the effect of GFP and GHP.	• Vaccination alone cannot guarantee the freedom of *Salmonella*. Therefore, good hygienic practices and biosecurity measures on pre-harvest, harvest and post-harvest level are essential.

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