Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Carrique-Mas, JJ; Davies, RH
Salmonella Enteritidis in commercial layer flocks in Europe: legislative background, on-farm sampling and main challenges
Revista Brasileira de Ciência Avícola, vol. 10, núm. 1, enero-marzo, 2008, pp. 1-9
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jan - Mar 2008 / v.10 / n.1 / 01 - 09

Salmonella Enteritidis in commercial layer flocks in Europe: Legislative background, on-farm sampling and main challenges

■ Author(s)

Carrique-Mas JJ
Davies RH

Department of Food and Environmental Safety
Veterinary Laboratories Agency

■ Mail Address

New Haw, Addlestone, Surrey KT15 3NB
Phone: +44-(0)1932-357814 (Ext. 2814)
Fax: +44-(0)1932-357595

Email: j.carrique-mas@vla.defra.gsi.gov.uk

■ Acknowledgments

The authors wish to thank Defra for funding through projects OZ0321, OZ322, and OZ0325. We are also grateful to Dr Felicity Clifton-Hadley (VLA) for her suggestions for improvement of the manuscript.

Arrived: March / 2008
Approved: May / 2008

BACKGROUND

During 2006 there were 160,645 reported human cases of salmonellosis in the then 25 Member States of the European Union (equivalent to an incidence of 35.4 cases per 100,000 population (EFSA, 2007a), making *Salmonella* the second most commonly reported gastrointestinal zoonotic infection across the EU. However, this figure is likely to be a considerable underestimate of the true incidence of disease. In the EU there are five serovars that account for the majority of cases of *Salmonella* in humans. These serovars are *S.* Enteritidis (SE), *S.* Typhimurium (ST), *S.* Infantis, *S.* Hadar, and *S.* Virchow, and were designated by the European Commission (EC) as 'serovars of public health significance'. However, in Europe, SE is by far the predominant serovar (59-62%), followed at a distance by ST (13-17%) (EFSA, 2007a; Fisher, 2004). The rise of SE over the last few decades can be rightly described as an epidemic. The number of human cases of SE started to increase dramatically in most countries of western Europe during the mid - to late eighties, and a similar phenomenon had been observed in some US regions a few years before (Saeed, 1999). This 'SE epidemic' involved mostly phage type (PT) 4, although in some European countries and the USA PT8 was initially predominant. In the UK, for example, at the peak of the SE epidemic (1996/97) PT 4 isolates made up 75% of all SE human isolates. Animal surveillance data suggested a rapid spread in commercial layers across the EU and beyond. The rapid spread of PT 4 worldwide suggests that the epidemic may have originated from infected grandparent breeding stock, and was subsequently amplified through hatcheries and disseminated globally. A notable exception to this phenomenon in western Europe was seen some Nordic countries with very strict *Salmonella* control programmes.

Epidemiological investigations demonstrated that contaminated eggs produced by infected laying hens were the main source of human infection with SE (Coyle *et al.*, 1988; Gillespie & Elson, 2005; Gillespie *et al.*, 2005; Rabsch *et al.*, 2001). SE shows an affinity for internal organs of the chicken and (in the case of laying hens) for the ovary and oviduct, leading to internal contamination of eggs prior to being laid (Guan *et al.*, 2006; Humphrey *et al.*, 1989; Poppe, 1999). However, the egg may also become contaminated in the shell when the poultry house environment is highly contaminated with SE. In the EU, washing of eggs is not permitted, and irradiation is not regarded as acceptable by the general population.

Because the European Union functions as a single market, the European Commission has played a major role in harmonising and co-ordinating monitoring and control programmes and ultimately aiming to reduce the prevalence of *Salmonella* in primary production of poultry across member states (MS). A key piece of EU legislation is the European Commission (EC) Regulation No. 2160/2003, which requires that MS

put in place control plans so that targets for the reduction of the prevalence of *Salmonella* at farm level can be achieved. A subsequent Directive (2003/99/EC) aimed at ensuring that zoonoses, zoonotic agents and their antimicrobial resistance are properly monitored, and that food-borne outbreaks are properly investigated. A number of initiatives followed, including baseline studies of *Salmonella* on poultry sectors across the EU. Earlier legislation (Directive 92/117/EC) had already established the principles of harmonised surveillance of chicken breeding flocks. In addition to this, specific legislation (Regulations (EC) No. 1774/2002 and (EC) No. 183/2005) laid down rules concerning animal by-products and vegetable feed materials.

The existing surveillance data from breeding flocks and baseline survey data from commercial chicken flocks were the basis of reduction targets on a country-by-country basis. These were to be achieved by the implementation of national control programmes (NCPs) for each sector in each country. The NCPs set out the monitoring procedures which are required to assess progress towards the target, as well as any measures which need to be taken to achieve the reduction.

Regulation (EC) No. 103/2005 contained specific reduction targets for *Salmonella* in flocks of breeding hens. Since 1 January 2007 flocks or hatcheries in every MS are to be sampled following one of two harmonised methods, designed to detect a within-flock prevalence of 1%. If SE or ST is detected as a result of this monitoring, hatching eggs can no longer be produced and the hens are culled or subjected to sanitary slaughter. If *S.* Infantis, *S.* Virchow, or *S.* Hadar is detected, the farmer needs to draft a specific action plan to eliminate infection and prevent dissemination. The target for each country is a maximum of 1% of breeding flocks infected by one of the five main serotypes. Third countries supplying hatching eggs or live poultry for breeding to the EU must have submitted a *Salmonella* control programme which is considered to be equivalent to the EU provisions. Sweden, Finland and Norway have additional import guarantees as a result of their superior *Salmonella* status in livestock production.

In the case of commercial layers, in order to provide the scientific basis for setting targets for reduction of the prevalence of *Salmonella*, an EU-wide *Salmonella* baseline survey was carried out on a randomised selection of commercial scale (>1,000 hens) laying farms (Commission Decision 2004/665/EC). This EU baseline survey was co-ordinated by DG SANCO and the European Food Safety Authority (EFSA). In each MS the survey was carried out by the veterinary authorities during 2004/2005. The specifications of this survey required the selection of a random sample of commercial laying holdings in each MS, stratified by the total number of hens on the holdings. From each holding only one flock was sampled within nine weeks of depopulation at the end of the laying period (Anonymous, 2004; Snow *et al.*, 2007). Results highlighted great variability in prevalence between MS. After the survey, results were considered by EFSA and the EC. Following this, annual reduction targets for SE and ST were set by Regulation (EC) No. 1168/2006 as a function of the prevalence based on the EU baseline survey. For countries with a SE and ST combined prevalence of <10%, an annual reduction of 10% was required. For countries with SE+ST combined prevalence of 10-19%, 20-39% and >39%, the required reductions were 20%, 30% and 40%, respectively. A further regulation (EC No. 1237/2007) brought forward trade restrictions on table eggs from flocks infected with SE or ST to 1st November 2007 in cases where the flock is the source of a *Salmonella* (any serovar) outbreak in humans and 1st January 2009, if the presence of SE or ST is demonstrated in flocks by routine monitoring.

Components of the NCPs in commercial layers

Regulation EC 1177/2003 laid down the minimal requirements for NCPs in commercial laying flocks. These were that: **1)** antimicrobials cannot be used to control *Salmonella*; **2)** Mandatory vaccination is to be used against SE of commercial layers in countries in which the SE prevalence is greater than 10%; **3)** live vaccines can only be administered to laying flocks in rear, and that the manufacturer must provide a method of distinguishing the vaccine from a field strain. The sampling programme of flocks is detailed in Commission Regulation (EC) No. 1168/2006. It involves testing flocks in rear by the producer: **1)** At day old (i.e. on arrival of the chick from the hatchery). This requires the testing of one chick box liner for every 500 chicks delivered from each hatchery; **2)** Two weeks before entering laying phase using two pairs of boot swabs (in non-cage rearing systems) per house or 60 1g faeces samples. The regulation also requires that the producer collects naturally two samples of pooled faeces (cage systems) or two pairs of boot swabs (non-cage systems), of all flocks during production, from the age of 22-26 weeks, and then every 15 weeks during the life of the flock. Samples are to be submitted for analysis to an

officially approved laboratory, where they are to be cultured using the ISO 6579:2002 (Annex D) method.

In addition, holdings with more than 1,000 laying hens are to be sampled by the competent authority. From each holding, every year one flock is to be randomly selected and two samples of faeces or two pairs of boot swabs (as described above) and one dust sample is to be collected. These samples are analysed in each country by the National Reference Laboratory or a suitable alternative using the same ISO 6579:2002 (Annex D) method. From February 2009, eggs from any flock detected as infected with SE or ST will be banned from the market unless they are heat treated. Because of the serious economic implications, a further regulation (EC No. 1237/2007) gives the producer the opportunity to dispute the findings from a positive result, if a 'false positive' result is suspected. This can be achieved by the collection and testing, at the producer's expense, of either of the following: **1)** seven faecal/environmental samples (as per the EU baseline survey); **2)** 4,000 eggs from the affected flock, analysed in pools of a maximum of 40 eggs (i.e. 100 pools); **3)** 300 birds tested for the presence of *Salmonella* in their caeca and ovary/oviducts (in pools of 5 of each type of tissue; i.e. totalling 120 pools). Negative results in these additional tests will overturn the original finding.

In summary, the implementation of standardized NCP with different targets of reduction according to baseline prevalence appears to be a sensible way to address the problem of SE in the laying sector across the EU as a whole. Some of the main relevant scientific and technical issues regarding sampling and detection are presented below, followed by a discussion about the main challenges faced facing the industry today.

Sampling for *Salmonella* in laying houses

Matrix of sampling: Faeces or dust?

The large number of competing bacteria is one of the major limiting factors in the isolation of *Salmonella* from faeces and other environmental samples. Environmental sampling of the poultry house is regarded as more cost-effective (Aho, 1992) and sensitive than sampling a limited number of individual birds (Davies & Wray, 1996a). In laying houses, a high level of environmental contamination of the house is also associated with a higher risk of producing contaminated eggs (Henzler *et al.*, 1998).

Faeces (especially if fresh) provide an indication of current infection of flocks, whereas contaminated dust may also indicate previous infection compared with faeces. Dust is however a more sensitive type of sample for detecting *Salmonella* in poultry flocks (Davies & Wray, 1996b; EFSA, 2007b). This is likely to be due to the comparative advantage of *Salmonella* in this type of matrix compared to other *Enterobacteriaceae*, which do not tend to survive as well in dry conditions (Haysom and Sharp, 2003).

The presence of large amounts of dust in the poultry houses may also be a hazard, since dust has been recognised as a vehicle of transmission of *Salmonella* when large numbers of organisms are present (Harbaugh *et al.*, 2006). *Salmonella* has been reported to survive in poultry houses at least 53 weeks in dust (Davies and Wray, 1996a) and up to 26 months in thin layers of litter, dried faeces and feed (Davies and Breslin, 2003b) after depopulation of a flock.

In laying houses, for example, sampling faeces alone may miss birds that have passed the peak of infection but which are still producing contaminated eggs (Riemann *et al.*, 1998). The sampling of both faeces and dust is recommended to increase the sensitivity of detection.

It is theoretically possible that positive faeces samples may only reflect transient infection in the birds, and that positive dust reflects contamination of vectors in the house (i.e. from infected rodents or flies) rather than current infection of the flock, but in our experience dust collected from the houses may become negative when an infected flock clears the infection.

The analysis of naturally pooled faecal material (i.e. originating from a large number of birds) is preferable to the analysis of a similar volume of individual droppings or cloacal swabs. Pooling faeces increases the chance of inclusion of material from infected birds which may be shedding high numbers of organisms, hence compensating for the typically low prevalence of infection (Arnold *et al.*, 2005). The maximum number of individual droppings that can theoretically be combined for culture without compromising the sensitivity of detection is still not well established for faeces from laying hens, but it is likely to be variable between flocks, depending on both the within-flock prevalence of *Salmonella*, its survival characteristics, the number of *Salmonella* organisms, and the ratio and type of competitor organisms present. In most cases, the sampling programme is determined by a combination of sensitivity and economic constraints. In situations of high prevalence it is likely that fewer samples would be necessary, but it is clear that a standardized protocol (with minor variations depending on the house type) is preferable in order to allow meaningful prevalence comparisons so that

epidemiological analyses can be carried out. Representative collection of faeces presents a challenge when flocks are in cages houses because of limited access, and it is particularly difficult in step cage systems when collection from the droppings pit is necessary.

It must be acknowledged that no sampling/testing method will achieve 100% detection sensitivity. The prevalence of infection and the number of organisms excreted may change over the life of a flock, and therefore sampling on repeated occasions during the life of a flock is likely to increase the chance of detection.

In non-cage (i.e. free-range and barn) houses boot swabs are normally used, since they can be used at the same time as routine inspection of the flock and can be more conveniently posted to the laboratory for analysis than litter. A study in Denmark showed that sampling broiler houses using five pairs of socks, cultured as pairs was at least as sensitive as sampling of 300 individual faeces samples (Skov *et al.*, 1999), and this was the basis for the adoption of the boot swab method in the EU surveys for non-cage houses. An important consideration when using boot swabs is to avoid contamination or contact with disinfectant (i.e. footbath) prior to use. The swabs should be moistened with a suitable diluent, normally potable water, to avoid problems of non-adherence if the manure/litter is dry. There is a wide range of commercial products that can be used as boot swabs. These include socks, surgical shoe covers, mob caps, that may vary in their absorbency, ease of fitting, and resistance to tearing. A concern with larger types of boot swabs is that they also require larger volumes of pre-enrichment medium (i.e. BPW) (McCrea *et al.*, 2006), which increases the cost of culture, as well as requiring more incubator space. In some countries drag swabs are used, which in their original form consisted of an assembly of at least three separate moistened 10x10cm surgical gauze swabs, attached to a string stapled to a wooden pole (Kingston, 1981; Mallinson *et al.*, 1989). Commercial adaptations of this include a pre-moistened cellulose sponge, which has limitations due to the small surface area and light weight. Studies in the US have shown that boot swabs performed similarly or better than drag-swabs when both were evaluated in parallel (Caldwell *et al.*, 1998; McCrea, 2005). It has recently been shown that stepping on drag swabs enhances the adherence of faeces and thus the rate of detection is increased compared with the standard dry swab methodology. However, the highest overall detection rate was still achieved using boot swabs (Buhr *et al.*, 2007).

In poultry houses accumulation of dust is common around air outlet vents, although it is often easier to collect it from horizontal surfaces such as partitions, ledges, and low beams. In many standard cage houses it is normally more practical to collect the dust from underneath the cages (Davies, 2005). In laying houses where automated egg collection is used dust can be also collected from the ends of the egg belts and from egg dust/spillage trays.

Collection and laboratory processing for EU baseline survey and NCPs samples

The specifications of the 2004/2005 *Salmonella* baseline survey required the collection of both faeces and dust from each flock. Faeces samples consisted of five 200-300g naturally pooled samples of faeces. To make up each sample, 20-40 pinches of faeces had to be collected from representative locations, which depended on the type of house. If the manure collection system involved belts or scrapers, faeces were to be collected from the scrapers or bars at the discharge ends of the cage rows. In 'A-frame' (step-cage) houses, faeces were to be collected from the deep pit. This is normally a challenging and unpleasant task. In non-cage housing systems, five pairs of boot swabs were to be collected, each of them from a representative 1/5 of the house. In cage flocks two samples of dusty material beneath cages (2 x 50g) had to be collected. In non-cage flocks one 50g sample of dust from egg belts and another 50g one had to be collected in different places of the house. Each sample has to be gathered from multiple separate locations.

For the NCP the method of collection of samples and processing were laid down in Commission Regulation (EC) No 1168/2006. NCP faeces (from cage systems), consist of two (i.e. one from each half of the house) 150g samples, which have to be taken from representative sampling points as described for the EU baseline survey. NCP dust samples consist of two (i.e. one from each half of the house) 50g samples. However sample processing is different for EU baseline survey and NCP. Whilst for the EU baseline survey each sample was analysed separately, in the case of NCP, samples are combined to produce a single result for each sample type (i.e. solid faeces, boot swabs or dust). This was achieved by a slightly different methodology in the laboratory. Each of the EU baseline survey faeces or dust sample was mixed with an equivalent volume of BPW, before adding 50g of this mixture to 200ml of

BPW for enrichment. In the case of NCP faeces or dust, the two samples are first combined and mixed together, and an aliquot (25g) is then added to 225ml of BPW.

EU baseline survey boot swab samples were processed by placing each pair into a honey jar containing 225ml BPW. In the case of the NCP boot swabs, the two pairs are placed together, and 450ml BPW is then added for pre-enrichment.

Preliminary unpublished observations on infected laying flocks in which both methods were compared suggested that on a per sample basis the NCP samples are at least as sensitive as the EU baseline survey samples. However, because of the small number of NCP samples taken per flock, the method as a whole has a lower sensitivity of detection, which may partially be compensated for by repeating the sampling every 15 weeks.

Collection of samples directly into BPW jars

An alternative to the collection of materials such as faeces, litter or dust, which requires processing in the laboratory, is the collection of faeces and dust using hand-held swabs, and taken directly into 225ml BPW jars. The swabs are autoclaved within the jars, making a ready-to-use sampling unit. A standardized method consisting of the collection 10 samples of dust and 10 samples of pooled faeces/litter from point locations in occupied laying houses was more sensitive than both the EU layer survey method and the NCP sampling methods, although on a per sample basis each sample is less sensitive than EU survey and NCP samples (unpublished data). By taking more samples from different locations the specific areas of contamination in the houses can be more precisely identified. Also each swab can be used to sample multiple sites, resulting in an increase in the per sample sensitivity, allowing a reduction in the number of hand-held swab samples taken. The jars of BPW containing the samples are directly incubated after arrival in the laboratory without the need for further processing. This 'wet swabbing' method is only suitable if samples can be quickly returned to the laboratory so that culture can begin soon after collection, and normally need to be taken by trained staff. Delaying the start of culture may result in *Salmonella* being overgrown by competitor organisms in the BPW.

This sampling method using BPW jars is particularly useful for assessing the efficacy of cleaning and disinfection (C&D) of poultry houses. This is because any residual disinfectant that may still be present on the surfaces is diluted by excess BPW. The highest rates of detection in breeder houses after C&D were observed in floor sweepings, nest box floors, slave hoppers, wall fabric junctions, feed troughs and high beams and pipes (Davies *et al.*, 2003; Davies & Wray, 1996a). In commercial layers in cage houses, drinkers/ drinker spillage cups, feeders, cage interiors, dropping belts / boards and floors can be sampled to assess C&D using this method (Wales *et al.*, 2006). It is useful to collect and test any rodent faeces or other pests observed in the house during this period to assess the risk of carry-over.

Main challenges to the control of *Salmonella* in commercial laying houses in the EU

The presence of SE in laying houses in the UK (and in many other EU countries) in most cases is probably a consequence of the historical introduction of this serovar with replacement birds during the period when parent breeding flocks were most likely to be infected. In these cases, SE has subsequently persisted over a number of production cycles without having been detected. In the UK, isolation of SE from other potential sources (i.e. feed, wild life) is currently rare (DEFRA, 2007) supporting the existence of a problem of persistent contamination in some commercial laying farms.

ST does not typically persist in laying houses for as long as SE (unpublished results), although from a legislative point of view, flocks affected with ST will be treated in the same way as those with SE. Because ST is much more common in wildlife, pigs and cattle, it has been postulated that free-range laying flocks will be at greater risk of becoming infected with ST than other production types. There is also a greater (albeit low) probability of introduction of ST via contaminated feed.

The long cycle of production (typically over a year), is an added difficulty for control, since the opportunities for interventions are limited during the laying phase of production of the flock.

In the UK, out of 15 houses that were detected to be positive with SE in the 2004/2005 EU baseline survey, 12 still remained contaminated with SE (in most cases with the same phage type also) when the follow-on flock was sampled (Carrique-Mas *et al.*, 2008). This is not surprising given that past monitoring programmes have not been very sensitive, and cleaning and disinfection standards and rodent control have not been adequate, partly as a consequence of market pressures.

In many EU countries, the egg industry has developed so that larger farms provide a range of grades of eggs from different ages of birds. This means that most large farms are continuously occupied with multi-age flocks, unlike breeding and commercial broiler production which is typically operated on an 'all-in-all-out' basis. The increased use of 'all-in-all-out' systems would have a positive impact on the control of *Salmonella* and other endemic diseases, but the economic costs are likely to make this impractical for some farmers. In some EU countries, induced molting of the hens is common practice in order to induce a second cycle of production, in which larger eggs are produced. Molting is known to increase the susceptibility of hens to SE (Holt and Porter, 1992).

Mice and rats represent a particular problem for laying farms. An association between the presence of rodents and infection of laying flocks with SE has been demonstrated (Garber *et al.*, 2003). Mice are highly susceptible to infection with SE (Davies and Wray, 1995a) and when the flocks are infected with *Salmonella*, rodent faeces collected from the house tend to be contaminated in a larger proportion than most other environmental samples (Davies and Wray, 1995b; Henzler *et al.*, 1998), since infected rodents are known to excrete a high level of *Salmonella* in their faeces (Davies and Wray, 1995c). It is thought that mice acquire infection through contact with faeces from infected birds (Taylor, 1956), but another contributing factor may be a horizontal transmission within colonies of mice (Welch *et al.*, 1941). Birds are thought to become infected after ingesting rodent droppings contaminating the feed. In EU baseline survey in the UK, there was a statistical association between the presence of rodents and infection with SE, whereas no such association was found for serovars other than SE (Snow *et al.*, 2008). Therefore in SE-infected farms, rodent control needs to be the priority.

Across EU cage systems are predominant, although they present a number of biosecurity challenges for the control of *Salmonella*. An important one is the presence of deep pits in some houses, particulary if the manure is left in the pit between flocks. In some cases the pit is not included in the disinfection programme, and is only removed by the farmer and used to fertilise land at the end of the summer cereal harvest.

A problem of multi-house cage sites is that there is often open communication between houses to give way to conveyor belts, feed pipes, passageways, etc. This close association of houses makes it easy for infection to be transferred from one flock to the other, although this is relatively rare in situations where there is absence of large populations of rodents or flies.

In addition, cage houses are intrinsically difficult to clean and disinfect to a good standard (Davies & Breslin, 2003a; Wales *et al.*, 2006). Cages are normally organised in 3-12 tier stacks with associated complicated structures including dropping boards/ belts drinkers, automatic egg belts, and feeder systems, Residual feed in particular may facilitate the multiplication of *Salmonella* after washing (Davies & Breslin, 2003a; Wales *et al.*, 2006). In many cases older houses have no drainage, and electrical systems may not be water-proof. Because of these limitations, some buildings have only been 'dry-cleaned', which is normally is not satisfactory to achieve elimination of *Salmonella*.

There are new EU welfare regulations planned that will impose the conversion of traditional cages into enriched-colony cage systems. In these systems, birds are allowed a relatively larger space with a scratching area, perches, a nesting area and a belt manure removal system, instead of a deep pit. However, the height of the multiple cage stacks also makes effective cleaning and disinfection a challenging task. The deadlines for the existing decommission of the existing cages is 2012, but at present it is not known whether this will be further delayed.

In most situations in which considerable levels of remaining organic matter are left after washing, only powerful disinfectants with a high penetrative power are capable of eliminating *Salmonella*. As a guide, disinfectants could be ranked in the following order of efficacy for dealing with high levels of *Salmonella* contamination in poultry houses: **1)** formaldehyde, **2)** glutaraldehyde or phenolics, **3)** quaternary ammonium based products; **4)** peroxygens and **5)** chlorine/iodine based disinfectants. Peroxygens would be normally more effective at high concentrations, but they may be corrosive for machinery and equipment. However, the application at the correct dilution rate is essential as well as the coverage of all disinfected areas to saturation point (Wales *et al.*, 2006).

Rodents take advantage of poorly maintained buildings with poor proofing and ample opportunity for harbourage. The deep pits typical of so many cage houses are ideal nesting grounds for rodents, but also the structure of the house (roof, insulation spaces, etc). Once established in a house, rodents can easily move from one house to the next, and therefore transmit infection between flocks. If populations of rodents exist

in the houses, it is very difficult to eliminate SE even if the visible surfaces of the buildings and equipment are successfully disinfected.

Dedicated disinfectant boot dips for each house, with regular replenishment of the disinfectant plus the use of disposable gloves and sanitisers should be standard practice in all laying farms, but unfortunately this is not followed in many cases. The maintenance of bio-security standards related to the entrance to houses is particularly important in non-cage houses, where the potential for spread of infection from bird to bird is greater. In these cases, it is preferable to use separate boots as no foot dip disinfectant can act quickly enough to guarantee total elimination of contamination from the boots.

Another important tool for control of *Salmonella* in layers is the use of vaccination. It is well known that vaccination reduces the risk of producing infected eggs by infected flocks (Davies and Breslin, 2004), but vaccinated hens are still likely to become infected when placed in houses with a previously infected flock and in which a deficient cleaning and disinfection was carried out, or when challenge from infected rodents is too high. There is a limitation in the level of protection against enteric bacteria that vaccines may confer. Live vaccines are very attenuated in order to minimise environmental survival. There may be an problem in giving the last dose of the vaccine too late (i.e. at transfer) given that in most cases newly placed pullets are challenged with *Salmonella* in the laying houses soon after transfer. There are also practical difficulties in the effective administration of vaccines, both for live and inactivated vaccines. It is important therefore to seek technical support from the vaccine companies when a vaccine is first used on a farm.

It is very important that vaccination does not promote a false sense of security, and lead to a relaxation in other necessary measures for successful control of *Salmonella*.

As previously mentioned, from February 2008, owners of flocks infected with SE or ST will not be allowed to sell Grade A eggs to the market across the EU. Because of this, many producers that are aware of infection of their flocks are making good progress, and in some cases they are successfully eradicating SE from the laying houses. Rapid elimination of SE within the life of a flock has been observed when the rodent populations have been eliminated.

CONCLUSIONS

The maintenance of *Salmonella*-free status can only be guaranteed if high standards of biosecurity and pest control are maintained. In situations where introduction of infection remains a moderate risk, vaccination is regarded as a tool which needs to be part of the overall control strategy. For houses where flocks have been detected with SE or ST, rodent control as well as an effective cleaning and disinfection programme at the end of flock is vital.

REFERENCES

Aho M. Problems of *Salmonella* sampling. International Journal of Food Microbiology 1992; 15:225-235.

Arnold M, Cook A, Davies R. A modelling approach to estimate the sensitivity of pooled faecal samples for isolation of *Salmonella* in pigs. Journal of the Royal Society Interface 2005; 2:365-372.

Baseline study on the prevalence of *Salmonella* in laying flocks of *Gallus gallus* in the EU. Official Journal of the European Union 2004; SANCO/34/2004 (Sept 30, 2004).

Buhr RJ, Richardson LJ, Cason JA, Cox NA, Fairchild BD. Comparison of four sampling methods for the detection of *Salmonella* in broiler litter. Poultry Science 2007; 86:21-25.

Caldwell DJ, Hargis BM, Corrier DE, DeLoach JR. Frequency of isolation of *Salmonella* from protective foot covers worn in broiler houses as compared to drag-swab sampling. Avian Diseases 1998; 42:381-384.

Carrique-Mas JJ, Breslin M, Snow L, Arnold ME, Wales A, McLaren I, Davies RH. Observations related to the *Salmonella* EU layer baseline survey in the United Kingdom: follow-up of positive flocks and sensitivity issues. Epidemiology Infectious 2008; 1-10.

Coyle EF, Palmer SR, Ribeiro CD, Jones HI, Howard AJ, Ward L, Rowe B. *Salmonella* enteritidis phage type 4 infection: association with hen's eggs. Lancet 1988; 2:1295-1297.

Davies R, Breslin M. Observations on *Salmonella* contamination of commercial laying farms before and after cleaning and disinfection. Veterinary Record 2003a; 152:283-287.

Davies R, Breslin M. Observations on *Salmonella* contamination of eggs from infected commercial laying flocks where vaccination for *Salmonella* enterica serovar Enteritidis had been used. Avian Pathology 2004; 33:133-144.

Davies R, Liebana E, Breslin M. Investigation of the distribution and control of *Salmonella* enterica serovar Enteritidis PT6 in layer breeding and egg production. Avian Pathology 2003; 32:225-237.

Davies R, Wray C. Mice as carriers of *Salmonella* enteritidis on persistently infected poultry units. Veterinary Record 1995a; 137:337-341.

Davies RH, Breslin M. Persistence of *Salmonella* enteritidis phage type 4 in the environment and arthropod vectors on an empty free-range chicken farm. Environmental Microbiology 2003b; 5:79-84.

Davies RH, Wray C. Contribution of the lesser mealworm beetle (*Alphitobius diaperinus*) to carriage of *Salmonella* enteritidis in poultry. Veterinary Record 1995b; 137:407-408.

Davies RH, Wray C. Determination of an effective sampling regime to detect *Salmonella* enteritidis in the environment of poultry units. Veterinary Microbiology 1996a; 50:117-127.

Davies RH, Wray C. Mice as carriers of *Salmonella* enteritidis on persistently infected poultry units. Veterinary Record 1995a; 137:337-341.

Davies RH, Wray C. Persistence of *Salmonella* enteritidis in poultry units and poultry food. British Poultry Science 1996b; 37:589-596.

Davies RH. Pathogen populations on poultry farms. Cambridge: Woodhead; 2005.

Departament of Enviroment, Food and Rural Affairs. *Salmonella* in livestock production in GB 2006. Reino Unido: Veterinary Laboratories Agency; 2007.

European Federation of Sea Anglers. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of *Gallus gallus* [cited 2008 jan]. Available from: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620761896.htm

European Federation of Sea Anglers. The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. The EFSA Journal 2007a; 130.

Fisher IS. Dramatic shift in the epidemiology of *Salmonella* enterica serotype Enteritidis phage types in western Europe, 1998-2003: results from the Enter-net international *Salmonella* database. Eurosurveillance 2004; 9:43-45.

Garber L, Smeltzer M, Fedorka-Cray P, Ladely S, Ferris K. *Salmonella* enterica serotype enteritidis in table egg layer house environments and in mice in U.S. layer houses and associated risk factors. Avian Diseases 2003; 47:134-142.

Gillespie I, Elson R. Successful reduction of human *Salmonella* Enteritidis infection in England and Wales. Eurosurveillance 2005; 10:E051117-051112.

Gillespie IA, O'Brien SJ, Adak GK, Ward LR, Smith HR. Foodborne general outbreaks of *Salmonella* Enteritidis phage type 4 infection, England and Wales, 1992-2002: where are the risks? Epidemiology and Infection 2005; 133:795-801.

Gradel KO, Andersen J, Madsen M. Comparisons of sampling procedures and time of sampling for the detection of *Salmonella* in Danish infected chicken flocks raised in floor systems. Acta Veterinaria Scandinavica 2002; 43:21-30.

Guan J, Grenier C, Brooks BW. *In vitro* study of *Salmonella* enteritidis and *Salmonella* Typhimurium definitive type 104: survival in egg albumen and penetration through the vitelline membrane. Poultry Science 2006; 85:1678-1681.

Harbaugh E, Trampel D, Wesley I, Hoff S, Griffith R, Hurd HS. Rapid aerosol transmission of *Salmonella* among turkeys in a simulated holding-shed environment. Poultry Science 2006; 85:1693-1699.

Haysom IW, Sharp K. The survival and recovery of bacteria in vacuum cleaner dust. Journal of the Royal Society of Health 2003; 23:39-45.

Henzler DJ, Kradel DC, Sischo WM. Management and environmental risk factors for *Salmonella* enteritidis contamination of eggs. American Journal of Veterinary Research 1998; 59:824-829.

Holt PS, Porter Jr RE. Effect of induced molting on the course of infection and transmission of *Salmonella* enteritidis in white Leghorn hens of different ages. Poultry Science 1992; 71:1842-1848.

Humphrey TJ, Baskerville A, Mawer S, Rowe B, Hopper S. *Salmonella* Enteritidis phage type 4 from the contents of intact eggs: a study involving naturally infected hens. Epidemiology and Infection 1989; 103:415-423.

Kingston DJ. A comparison of culturing drag swabs and litter for identification of infections with *Salmonella* spp. in commercial chicken flocks. Avian Diseases 1981; 25:513-516.

Mallinson ET, Tate CR, Miller RG, Bennett B, Russek-Cohen E. Monitoring poultry farms for *Salmonella* by drag-swab sampling and antigen-capture immunoassay. Avian Diseases 1989; 33:684-690.

McCrea BA, Macklin KS, Norton RA, Hess JB, Bilgili SF. A longitudinal study of *Salmonella* and Campylobacter jejuni isolates from day of hatch through processing by automated ribotyping. Journal Food Protection 2005; 69:2908-2914.

McCrea BA, Norton KS, Macklin JB, Hess JB, Bilgili SF. Recovery and genetic similarity of *Salmonella* from broiler house drag swabs versus surgical shoe covers. Journal Applied Poultry Research 2006; 14:694-699.

Poppe C. Epidemiology of *Salmonella* enterica serovar enteritidis. Ames: Iowa State University Press; 2005.

Rabsch W, Tschape H, Baumler AJ. Non-typhoidal salmonellosis: emerging problems. Microbial Infection 2005; 3:237-247.

Riemann H, Himathongkham S, Willoughby D, Tarbell R, Breitmeyer R. A survey for *Salmonella* by drag swabbing manure piles in California egg ranches. Avian Diseases 1998; 42:67-71.

Saeed AM. *Salmonella* enterica serovar enteritidis in humans and animals: Epidemiology, pathogenesis and control. Ames: Iowa State University Press; 1999.

Skov MN, Carstensen B, Tornoe N, Madsen M. Evaluation of sampling methods for the detection of *Salmonella* in broiler flocks. Journal Applied Microbiology 1999; 86:695-700.

Snow LC, Davies RH, Christiansen KH, Carrique-Mas JJ, Cook AJ, Evans SE. Investigation of risk factors for *Salmonella* on commercial egg-laying farms in. Great Britain 2004-2005; 2008.

Snow LC, Davies RH, Christiansen KH, Carrique-Mas JJ, Wales A, O'Connor JL. *et al.* Survey of *Salmonella* prevalence in Laying flocks in the United Kingdom. Veterinary Record 2007; 161:471-476.

Taylor J. Bacterial rodenticides and infection with *Salmonella* enteritidis. Lancet 1956; 270:630-633.

Wales A, Breslin M, Davies R. Assessment of cleaning and disinfection in *Salmonella*-contaminated poultry layer houses using qualitative and semi-quantitative culture techniques. Veterinary Microbiology 2006; 116:283-293.

Welch H, Ostrolenk M, Bartram MT. Role of rats in the spread of food poisoning bacteria of the *Salmonella* group. American Journal of Public Health Nations 1941; 31:332-340.

PUBLICAÇÕES FACTA

Anais da Conferência APINCO'94

Anais da Conferência APINCO'95

Anais da Conferência APINCO'97

Anais da Conferência APINCO'98

Anais da Conferência APINCO'99 (2 vol)

Anais da Conferência APINCO'00 (2 vol)

Anais da Conferência APINCO'02 (1 vol)

Anais da Conferência APINCO'03 (1 vol)

Anais da Conferência APINCO'05 (2 vol)

Anais da Conferência APINCO'06 (1 vol)

Anais da Conferência APINCO'07 (1 vol)

II Simpósio Internacional de Coccidiose Aviária

IX Conferência Internacional de Coccidiose Aviária

IX Conferência Internacional de Coccidiose Aviária (CD)

Col. FACTA - Manejo de Frangos

II Simpósio da Doença de Gumboro

Simpósio FACTA de Postura Comercial

Doenças das Aves

Manejo da Incubação

Produção de Frangos de Corte

Manejo de Matrizes de Corte

Para maiores informações: