
CHAPTER 11

Salmonella infection in poultry: the production environment

C. Wray, R.H. Davies and S.J. Evans

Central Veterinary Laboratory, New Haw, Addlestone KT15 3NB, UK

INTRODUCTION

More than 2300 different salmonella serovars have been described and although all members of the species are considered to be potentially pathogenic, they differ widely in their host range and pathogenicity. In the veterinary literature a distinction is usually made between infection caused by the two host-adapted serovars, *Salmonella pullorum* (pullorum disease) and *S. gallinarum* (fowl typhoid), the arizona group of salmonellas (arizonosis) and the remainder of the salmonellas (salmonellosis, paratyphoid infection).

Pullorum disease and fowl typhoid were the subject of government-backed control schemes in the United Kingdom and are now infrequent causes of disease. However, in many other countries they are of major importance, resulting in considerable economic losses. They are, however, of little zoonotic importance and will not be considered further.

Paratyphoid infections, generally subclinical, are common in domestic poultry throughout most of the world. Many different serovars have been identified in domestic poultry and one particular serovar may predominate for a number of years before it is replaced by another. Thus, in 1943, *S. thompson* appeared in the UK poultry flock and within two years was the most frequent salmonella isolated from poultry. In the 1970s, *S. agona* was introduced into the country in imported Peruvian fish meal (Turnbull, 1979) and became widespread in poultry and subsequently humans. Likewise, *S. hadar*, first recorded in 1969, affected the turkey industry because it caused disease in table birds which led to food-borne illness in humans (Watson and Kirby, 1984).

During the late 1980s there was a dramatic increase in *S. enteritidis* in poultry and since 1987 this has been the most frequent serovar isolated from poultry in the UK (Fig. 11.1). The increase was associated with the emergence of phage type 4 and a corresponding rise in human illness, with many food poisoning outbreaks having been attributed to poultry products. A similar increase in the prevalence of *S. enteritidis* in both humans and poultry has been observed in many other countries although, in some instances, it has been caused by phage types other than 4, e.g. phage type 13a in the USA. Some countries, such as Australia, have remained free from *S. enteritidis*. Likewise, *S. typhimurium* is another important serovar in poultry (Table 11.1)

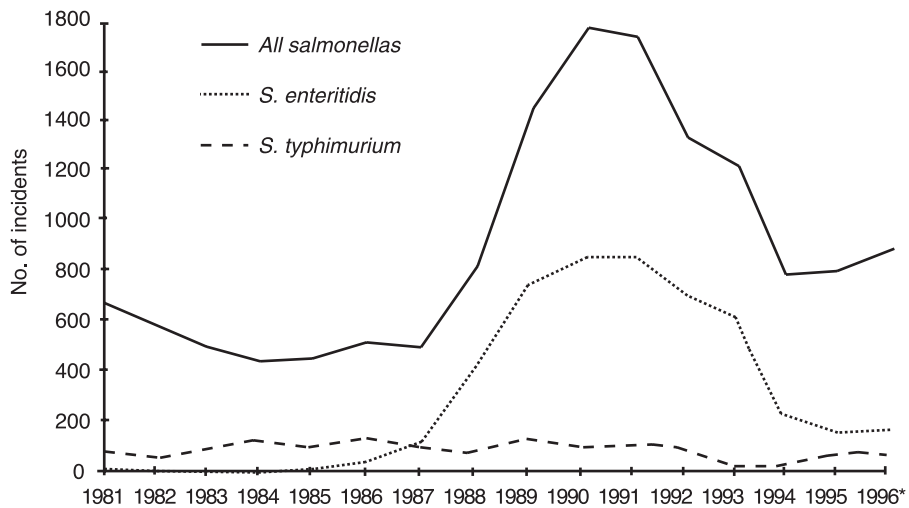


Fig. 11.1. *Salmonella* incident reports in Great Britain (1981–1996). *Provisional figures. Source: MAFF.

and currently the presence of DT104, which is often resistant to a number of antimicrobials, is giving cause for concern.

Efforts to control salmonella infections in poultry are, with the exception of those caused by pathogenic serovars, driven by public health considerations rather than expectations that dramatic improvements in production efficiency will be achieved. To this end, the Zoonoses Directive (92/117) aims to control the presence of *S. enteritidis* and *S. typhimurium* in breeder flocks and so prevent vertical transmission to the commercial sector.

Table 11.1. Number of incidents of *S. enteritidis*, *S. typhimurium* and other salmonellas reported under the Zoonoses Order 1989 for the period 1 January–31 December 1996 compared with the same period in 1995.

	<i>S. enteritidis</i>		<i>S. typhimurium</i>		Other <i>Salmonella</i> spp.	
	1995	1996	1995	1996	1995	1996
Broilers	72	98	48	61	337	431
Broiler breeders	66	45	16	9	208	210
Layers	11	13	5	3	9	11
Layer breeders	6	6	2	1	19	22
Turkeys, ducks and geese	46	16	100	97	232	219
Other birds	27	32	83	70	118	189
Total birds	228	210	254	241	923	1082

ORIGINS OF INFECTION

Once a salmonella with an affinity for poultry has become established in a primary breeding flock, it can infect poultry in other units via hatcheries by both vertical and lateral spread. This can have far ranging and serious effects on the health of both poultry and humans. It is thus of considerable importance to establish how infection can be introduced into a salmonella-free breeding flock given the complex epidemiology (Fig. 11.2).

The poultry industry is separated into egg and meat production enterprises, each of which has its own breeding flock hierarchy. Elite breeder flocks contain the primary genetic stock, whose offspring form the grandparent flocks which, in turn, produce parent breeder flocks. Involvement

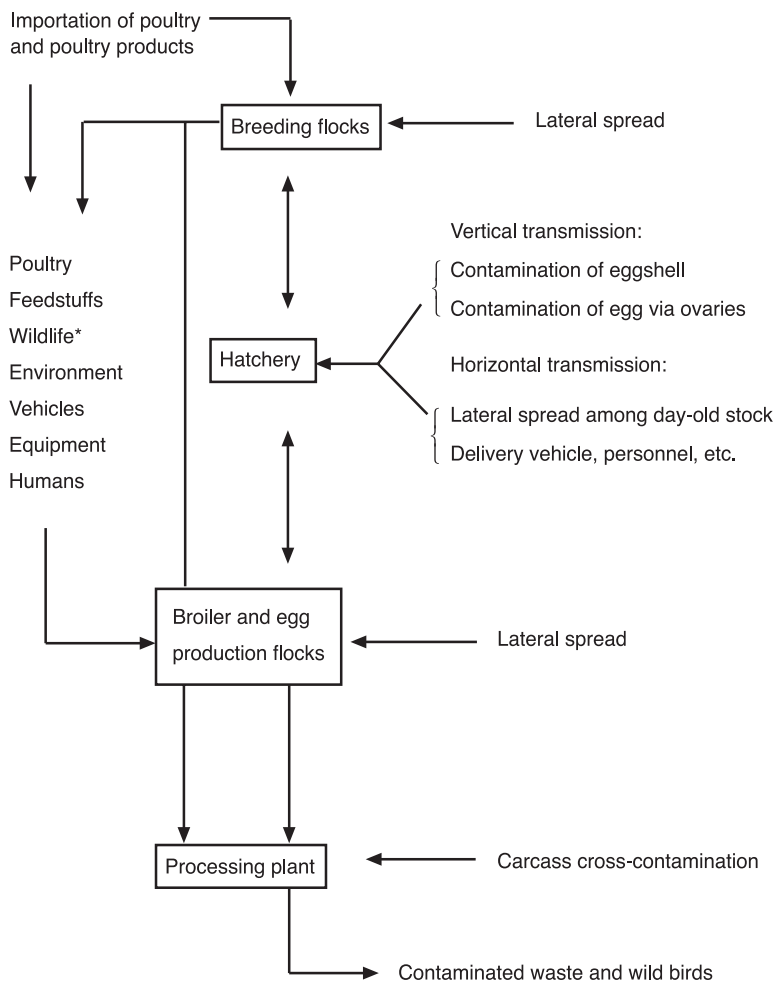


Fig. 11.2. Cycle of *Salmonella* infection. *Wildlife includes vermin, wild birds and insects.

of breeding and production flocks from both sectors of the British Poultry Industry, and the ability of *S. enteritidis* to be transmitted vertically to offspring, may partly explain the widespread nature of the epidemic (O'Brien, 1988; Lister, 1988). However, there have been no confirmed flock infections with *S. enteritidis* in elite or grandparent breeding flocks since the start of compulsory MAFF (Ministry of Agriculture Fisheries and Food) monitoring for salmonella in 1989.

If a breeding flock is infected with salmonellas, a cycle can be established by which the organism passes via the eggs to the progeny and even to chicks hatched from eggs laid subsequently by infected progeny. This cycle can occur by true ovarian transmission, infection within the oviduct or, as is much more likely to happen, through faecal contamination of the egg surface. As the egg passes through the cloaca, salmonella in faeces attach themselves to the warm, wet shell surface and may be drawn inside as it cools. Surface contamination may also occur in the nest boxes.

The possibility of trans-shell invasion of hatching eggs by salmonella is of great concern. Experimentally, substantial trans-shell infection of eggs has been demonstrated, following application of aerosols of salmonella or contact with contaminated litter (Mario-Padron, 1990). Nest box hygiene to reduce contamination and rejection of floor eggs is particularly important to reduce such surface contamination of eggs, which also carries a risk of introducing contamination into the hatchery environment and to personnel and equipment (Bruce and Drysdale, 1990). Damage to the cuticle of the eggshell is important because it allows increased penetration of organisms (Haigh and Betts, 1991). Such damage may occur as a result of commercial handling conditions (Nascimento *et al.*, 1992) or during certain washing procedures (Mayes and Takeballi, 1983). Poor quality shell resulting from adverse nutritional factors or stress may also enhance trans-shell infection (Nascimento and Solomon, 1991).

THE HATCHERY

Modern hatcheries, which are highly automated and have a high throughput of eggs and chicks, are complex operations where many factors interrelate to influence the prevalence of salmonella contamination. Hatcheries can serve as reservoirs of infection and cross-contamination in the hatchery may dramatically increase the prevalence of salmonella-infected chicks leaving the hatchery when compared with the low prevalence of infected eggs entering the hatchery. During the incubation of infected eggs, there is a rise in the number of salmonellas within the egg and in the detectable infection rate (Cason *et al.*, 1991; Hammack *et al.*, 1993). This has no apparent effect on hatchability, so there may be potential for amplification of contamination as salmonellas are released during the hatching process. Bailey *et al.* (1994) demonstrated that a single salmonella-contaminated egg could substantially contaminate other eggs and chicks in the hatching cabinet.

A comparative study of salmonella contamination in 11 commercial hatcheries indicated the main cross-contamination hazard points, but significant reductions in contamination were achievable by good organization, hygiene management and disinfection practices (Davies and Wray, 1994c).

The most important factor in salmonella contamination of a hatchery is the infection status of flocks supplying the eggs. As part of our own studies two hatcheries were sampled before and after elimination of known *S. enteritidis*-infected supply flocks. In both cases, there was no evidence of current *S. enteritidis* contamination within the hatchery at the second visit, although other serotypes were present. In a well-run hatchery there should be little opportunity for long-term persistence of salmonellas at a level that would pose a significant threat to chicks in the absence of an infected egg source. However, if there is potential for high level cross-contamination of eggs and chicks or poor disinfection of incubator ventilation systems, salmonella infection may be perpetuated in future chick crops.

Egg sanitization is the first barrier to the introduction of microbial contamination into the hatchery premises via the egg surface. Egg sanitization on the farm, even where disinfectant fogging was used in the hatchery, was found to be insufficient and further egg treatment by formaldehyde vapour fumigation or further egg sanitization through a well regulated wash machine was required. It was often possible to observe poor removal of litter, feathers and faeces from eggs at certain breeder farms.

Setter incubators are traditionally considered to be low-risk areas for salmonella multiplication and cross-contamination, since eggs are removed before hatching takes place. However, some bursting of eggs and premature hatching does occur. In some hatcheries, there was a high prevalence of salmonella isolation in setters which was associated with poor cleaning due to fixed tray-turning apparatus and failure to remove old shell debris. Fogging with disinfectant was commonly carried out, but was not effective where physical cleaning was poor. More aggressive physical removal of egg debris and disinfection of surfaces were needed in many cases. The system showing least salmonella contamination was single-stage setting which allowed all-in, all-out handling of eggs with effective sanitization between batches. Control of salmonella contamination in setters is important because the warm, humidified air may disseminate salmonella over the surfaces of several batches of eggs within the same incubator.

After 18 days of turning in the setter incubators, the eggs are transferred to hatcher incubators for the final three days of incubation. The transfer from egg trays to hatching baskets is often semiautomatic, using multiple suction cup machines which transfer a whole tray of eggs in one operation. This speeds up the process but the suction heads are sometimes contaminated with salmonellas and so could cause cross-contamination of different batches of eggs. In addition, there appears to be a higher prevalence of broken eggs with some automatic transfer machines so that the surrounding area becomes more contaminated. It is obviously uneconomic to transfer eggs manually in large hatcheries, but tray-turning and emptying devices may be used with advantage in some circumstances. Frequent and effective disinfection of

surfaces and suction cups of egg-transfer machines has resulted in improvements in salmonella contamination rate.

Hatching of eggs liberates large quantities of dust and fluff from the chicks which may be highly contaminated with salmonellas (e.g. 10^4 g⁻¹) if eggs from an infected flock are being hatched. The organisms may be circulated within the hatcher by the ventilation system. Chicks from more than one flock may be placed in the same hatcher because of the need to maintain fully stocked incubators and ensure stable incubation conditions. In relation to the high throughput, the hatchers have a larger capacity, but are few in number. This situation may lead to cross-contamination of chicks from different supply flocks.

Effective use of formaldehyde vapour during hatching can be demonstrated, but it is common to find insufficient volumes of formaldehyde solution, irregular replenishment of the solution or formaldehyde containers of insufficient surface area to permit effective vaporization. These errors may allow multiplication of salmonellas in the hatchers.

In many cases, the ventilation system of the hatchers discharges contaminated air into either a common airspace or a poorly sealed dust trap corridor or loft. There are also instances where the air intake fans of pressurized hatchers discharge chick dust to the exterior. This may lead to contaminated air being drawn into the other hatchers in the same airspace, so that chicks from salmonella-free flocks may be at risk from salmonella cross-infection while in the hatcher. As hatching occurs over a 3-day period (days 18–21) there may be ample opportunity for excretion and multiplication of salmonellas before chicks are removed from the hatcher.

Following hatching, chicks are sorted, vaccinated and packed in delivery boxes or crates. When chicks from infected flocks are handled, there is the potential for salmonella contamination of the largely automated handling equipment and its environment via meconium and fluff. If a salmonella-infected flock is handled before a non-infected flock there is a risk of surface cross-contamination of chicks, with subsequent oral infection during preening. It is important to organize all hatchery operations so that eggs and chicks from potentially the least contaminated sources are handled first or eggs from flocks that are known to be infected are hatched on separate days. In some cases present hatchery organization does not allow this degree of prioritization.

The sanitization of chick handling equipment is important to avoid carry-over of salmonella contamination from day to day. In our own investigations, there have been examples of good and poor cleansing and disinfection practices. It is important to remove gross debris before pressure washing, preferably by liquid vacuum suction. The surfaces should then be washed with a detergent sanitizer applied by a power washer set at medium pressure whilst the conveyors are running. Ledges and inaccessible areas should be wiped with disposable cloths soaked in disinfectant and finally all surfaces should be allowed to dry before being sprayed at low pressure with an effective disinfectant applied at the correct concentration for a high-risk salmonella contaminated area (MAFF General Orders rate).

Effective sanitization of egg trays, chick trays and farm trolleys is necessary to avoid carry-over of infection between batches of eggs or chicks. Total elimination of salmonella is particularly important for trays and trolleys that are returned to breeder units, as these may otherwise introduce salmonella from the hatchery to previously uninfected premises.

The efficacy of tray washing appears to vary. Some sanitized trays appear extremely clean whereas others may be contaminated with particles of eggshell, yolk or meconium. It is possible to achieve salmonella-free trolleys and trays using either manual washing or automatic tray washers, but the latter must be set up correctly to clean effectively. The type and concentration of disinfectant used in tray washing also appears to be an important factor, since visibly clean trays can still harbour infection on some occasions.

In some hatcheries, the main ventilation system draws air from areas with a potential source of salmonella contamination, such as the hatcher and chick area, air exhaust ducts or the waste skip, where splashing of macerated egg and dead chick remains can occur. Where such hazards are present, salmonella is always likely to be found in air intake ducts. Because these ducts are high-speed intakes there can be very little dust to sample, so the presence of salmonellas may reflect a larger number of organisms drawn back into the building to be distributed to a variety of areas. In many cases, coarse filtration of air is used, but this is unlikely to restrict the access of small, contaminated dust particles.

In most cases, it would be possible to upgrade the filtration of air. Effective screening of the air intake plant is also beneficial and spread of contamination from exhaust ducts can be reduced by the use of sanitary traps. Ideally, all hatcheries should be designed so that air is drawn in from the opposite side of the building to that on which stale air and waste are discharged. Similarly, recirculation of air to conserve heat should be discouraged unless effective bacteriological filters are used.

There are many other areas such as transport vehicles, personnel, chick-holding accommodation, waste management, segregation of 'dirty' and 'clean' areas, avoidance of backtracking and personal hygiene measures and facilities which are also important in controlling salmonella contamination in hatcheries.

ENVIRONMENTAL CONTAMINATION

Persistent environmental contamination of houses is an important factor in the maintenance of *S. enteritidis* and other salmonellas in poultry flocks (Kradel and Miller, 1991; Baggesen *et al.*, 1992). The effective decontamination of salmonella-infected houses before repopulation is a highly important consideration in a Hazard Analysis Critical Control Point approach for poultry units. A high standard of disinfection is necessary to avoid infection of poultry placed in previously infected houses, because it has been shown experimentally that an infective dose of salmonella for chickens can be less than five cells (Milner and Shaffer, 1952) or 100 cells for adult birds following conjunctival inoculation (Humphrey *et al.*, 1992). Intercurrent disease may

make the birds even more susceptible (Arakawa *et al.*, 1992; Holt, 1993; Nakamura *et al.*, 1995). A number of analytical studies have associated salmonella infection with poor hygiene standards at poultry sites (Opitz, 1992; Henzler and Opitz, 1992; Fris and van den Bos, 1995). The tendency for persistent infection on the farm is widely recognized and in a case control study of British poultry breeding flocks, *S. enteritidis* PT4 infection was associated with a history of salmonella at the poultry site, highlighting the importance of the farm environment in the epidemiology of infection (Evans and Sayers, unpublished observations). Similar findings were reported following a study of broiler flocks in Denmark (Angen *et al.*, 1996).

Salmonellas may persist in dry livestock buildings for many months (Bailey, 1993; Bale *et al.*, 1993) and our own studies (Davies and Wray, 1996a) have confirmed the ability of these organisms to survive for long periods (Table 11.2). Samples were obtained for a 12-month period from a poultry house, which had contained birds naturally infected with *S. enteritidis*. There was a high isolation rate of salmonella from samples taken two weeks before depletion of the flock, particularly at ground level and in nest boxes. One week after removal of all the birds, no salmonellas were found in litter or droppings picked from the litter surface, although feed troughs, nest boxes and other areas were still contaminated. The rapid decline in prevalence of salmonella isolations from litter and faeces suggests that continued deposition of salmonellas from infected birds may be necessary to maintain contamination of litter. The prevalence of salmonellas remained low until the removal of the litter at 30 weeks, when 41% of the 36 swabs taken from the floor were contaminated with salmonellas (Table 11.2), even though salmonellas were not isolated from bulk litter removed from the house. However, the organism was present in small pockets of spilt litter, which remained outside the house after depletion. Similar results were obtained from two broiler breeder sites, where salmonellas were detected in fan dust outside the house, whereas swabs taken within the house were negative. Further studies in occupied poultry houses (Davies and Wray, 1996b) found a threefold higher salmonella isolation rate from nest box floors and dust on in-house slave feed hoppers than from drinkers, chain feeders, slats, perches or dust on beams and ventilation ducts. In broiler breeder houses, salmonellas were isolated from egg sorting tables and 75% of the egg collecting trolleys that were sampled.

Table 11.2. Persistence of *S. enteritidis* in an empty poultry unit.

Sample site	Two weeks before depletion	After litter removal at 30 weeks after depopulation
Drinkers	3/4 ^a	0/3
Ground swabs	4/4	15/36
Litter	4/4	0/20
Walls	3/4	4/12
Dust on beams	2/4	2/12
Total	16/20 (80%)	21/83 (25.3%)

^aNo. of *Salmonella*-positive samples/total no. of samples.

DISINFECTION STUDIES

A high standard of disinfection is necessary to avoid infection of poultry placed in a previously infected house and studies carried out at the Central Veterinary Laboratory and in the field have identified many potential problems during disinfection of poultry units that were naturally contaminated with *S. enteritidis* (Davies and Wray, 1995a). Sampling carried out on cleaned and disinfected poultry houses after infected flocks had been slaughtered, showed persistence of *S. enteritidis* in the environment of 16 of 20 houses. Cleansing and disinfection regimens using formaldehyde, either as part of a terminal disinfectant spray strategy or as a fogging agent after the use of other products, were associated with the lowest level of persistent salmonella contamination. Thus, *S. enteritidis* was not found to have persisted in five of the nine houses in which a formaldehyde disinfectant spray or fogging treatment had been used. In another six of the houses, *S. enteritidis* was only found on equipment that had not been treated with formaldehyde, and in another house only 1 of 90 samples was positive. The other two houses in which formaldehyde had been used were heavily reinfested by *S. enteritidis*-infected mice. There appeared to be a relationship between the standard of cleansing and the level of persistent salmonella when a tar-oil mixture spray and a peroxygen compound fog was used. However, when a synthetic phenolic compound was used as a spray, salmonellas were not isolated from treated surfaces, even in the presence of large quantities of organic matter and the organism was only detected on equipment that had not been disinfected.

The prevalence of salmonella contamination was significantly increased in a contaminated house following ineffective cleansing and disinfection (Table 11.3). Kradel and Miller (1991) also observed increased contamination leading to persistent poultry flock infection following environmental carry over of salmonella.

The poor results of some cleansing and disinfection regimens lend support to those that believe in leaving poultry litter *in situ*, which is claimed to increase colonization resistance of chicks to salmonella (Corrier *et al.*, 1993), but which may lead to a build up of a wide range of harmful organisms and degrade the principle of all-in, all-out stocking as a means of breaking disease cycles.

Table 11.3. Persistence of *S. enteritidis* in a broiler house after cleansing and disinfection.

Sample site	Before sanitization (%)	After sanitization (%)
Floor	4/7 ^a (57.0)	6/8 (75.0)
Walls	0/5 (-)	4/7 (57.1)
Post bases	0/5 (-)	5/8 (62.5)
Chain feeders	1/8 (12.5)	3/6 (50.0)
High beams and pipes	1/6 (16.7)	0/8 (-)
Total	6/31 (19.3)	18/37 (48.6)

^aNo. of *Salmonella*-positive samples/total no. of samples.

It was generally observed that the standard of cleaning of poultry houses by farm staff was inferior to that of contractors (Table 11.4). The level of supervision by site and field management was low, resulting in missed areas and in errors that could compromise the disinfection of the site.

Further work is necessary, however, to confirm that these regimes are effective over a wide range of situations. Use of disinfectants, especially formaldehyde, necessitates that all safety requirements are met, especially the use of a respirator to protect the operator. Although effective cleansing and disinfection carries a cost, improvements in broiler growth rates have been reported in formaldehyde-treated houses (Allen, 1993).

THE ROLE OF WILDLIFE

Elimination of the persistent contamination of some poultry breeder units has been one of the most difficult problems in controlling *S. enteritidis* and other salmonella serotypes in poultry flocks in Great Britain and other countries (Baggesen *et al.*, 1992; Brown *et al.*, 1992). Such persistent contamination may be caused by failure of disinfection routines or the presence of wildlife carriers or vectors.

Role of Rodents

Although *S. enteritidis* infection in mice on poultry units was reported more than 15 years ago (Krabisch and Dorn, 1980), the significance of mice as vectors of *S. enteritidis* on poultry units has only received widespread attention relatively recently (Henzler and Opitz, 1992). Naturally infected mice, captured at depletion on poultry units where *S. enteritidis* infection had been confirmed in the birds, excreted the organism for up to 18 weeks (Davies and Wray, 1995b). Excretion was intermittent and reactivation of infection occurred during periods of stress. The prevalence of *S. enteritidis* in individual faecal pellets was usually low (< 10 colony-forming units (cfu)) but one pellet contained 10^2 – 10^3 organisms. More recent work has identified levels of 10^5 – 10^6 cfu in some faecal pellets. Salmonella contamination in the environment may be amplified by mice defecating into feed troughs and on egg-collection belts and may be spread further throughout the house by automated feeding systems, egg conveyors and manure removal equipment.

Table 11.4. Persistence of *Salmonella* after cleansing and disinfection.

No of units	Application by	No. of samples positive/ total no. of samples (%)
7	Farm staff	185/1238 (14.9)
13	Contractor	143/1921 (7.4)

S. enteritidis-infected mice were detected in a single poultry house for more than two years after depopulation and they constituted a reservoir of infection for the next flock. Infected, dead mice or droppings were found on 50% of broiler breeder or layer breeder units that were investigated after cleaning and disinfection. Many areas on poultry units may become infected with rodents and an intensive and sustained rodent control programme is necessary for the control of salmonella. The programme needs to be well planned, flexible, continuous and its effectiveness monitored (WHO, 1994). Trapping of rodents may also be used to monitor salmonella contamination, because mice remain infected even after environmental contamination becomes difficult to detect by standard sampling techniques.

Role of Birds

Salmonella infection has been detected in many species of wild bird. At hatcheries and poultry-processing plants salmonellas were detected in a number of different species of wild birds, which may contaminate clean equipment left outside the buildings (Davies and Wray, 1994a).

Role of Insects

Flies have frequently been shown to be contaminated with salmonella and Edel *et al.* (1973) found that 1.5% of 202 fly traps examined were contaminated with the organism. Blowfly larvae (*Lucilia serricata*) were also found to be contaminated with salmonellas and our studies have shown that maggots are a potent vehicle of salmonella infection for chickens (Davies and Wray, 1994b). Maggots, which may contain up to 10^6 cfu of salmonella depending on the substrate, are attractive to chickens and, when ingested, the cuticle has a protective effect so that the bactericidal activity of gastric acidity etc. is by-passed.

It has been suggested that mealworm beetles (*Alphitobius diaperinus*) may also be important in the persistence and transmission of salmonella infections on poultry units (Baggesen *et al.*, 1992; Brown *et al.*, 1992). In our studies 500 live *Alphitobius* beetles were collected before cleansing and disinfection on two poultry units, and although the environmental contamination with salmonella was high the organism was not isolated from any of the beetles. Likewise we failed to infect the beetles by artificial contamination with 10^3 cfu of salmonella, although Geissler and Kösters (1972) found that beetles artificially infected with 10^9 cfu excreted salmonellas for 15 days.

BIOSECURITY

Staff on farms and visitors can carry salmonellas mechanically from one unit to another on contaminated equipment, footwear, clothing and hands. As a consequence, visitors to livestock units should be restricted to those on

essential business and adequate protective clothing should be provided and hygienic procedures adhered to.

The farm should be located away from other poultry holdings, where circumstances permit, and visitors should park away from the buildings, preferably outside the holding. No visitor should enter a poultry building unless wearing disposable overall clothing, or overall clothing which is capable of being laundered and boots which are capable of being cleansed and disinfected. On leaving a poultry building, the person should immediately cleanse and disinfect boots and wash hands. Further details of biosecurity may be found in the Codes of Practice for the prevention and control of salmonella in poultry flocks (MAFF).

STATUTORY ASPECTS OF THE CONTROL OF SALMONELLA IN GREAT BRITAIN

In 1989, a new Zoonoses Order replaced and broadened the scope of the previous order which was first enacted in 1975. The main provisions of the Zoonoses Order (HMSO, 1989a) are the requirement to report the results of tests which identify the presence of salmonella, the provision of a culture of the salmonella for MAFF, the taking of live birds and other samples for diagnostic purposes, imposition of movement restrictions and isolation requirements, as well as a requirement for the cleaning and disinfection of premises and vehicles. The Order also applies the provision of the Animal Health Act (HMSO, 1981a) with regard to the compulsory slaughter of salmonella-infected poultry flocks and payment of compensation.

To combat *S. enteritidis* infection in poultry, the Poultry Breeding Flocks and Hatcheries (Testing and Registration) Order was enacted in 1989 (HMSO, 1989b). Both orders required the testing of poultry for salmonella on a regular basis. The purpose was to prevent transmission of salmonella through eggs and to reduce vertical transmission of salmonellas so that chickens for commercial rearing did not take infection on to customers' premises. The two orders were revoked in 1993 with the implementation of the Poultry Breeding Flocks and Hatcheries Order (HMSO, 1993) which brought salmonella control measures in poultry into line with the European Union Directive 92/117/EEC. This requires the regular monitoring of breeding flocks and hatcheries for *S. enteritidis* and *S. typhimurium* by a prescribed programme using methods laid down in the Order.

Since 1993, there has been no statutory requirement to monitor turkeys, ducks or geese, or the commercial generations of domestic fowl. Flock owners have, however, been encouraged to adopt good management practices for the control of salmonellas by following voluntary codes of practice that have been produced by the Ministry in collaboration with the poultry industry and the veterinary profession.

SALMONELLA IN ANIMAL FEEDS

Feedstuffs have always been a potential source of salmonella for poultry and the Processed Animal Protein Order (HMSO, 1989c) requires those processing animal protein to be registered with the Ministry and to test each day's consignment for salmonella in an authorized laboratory. If salmonella is isolated the processor is required to ensure that no contaminated material is incorporated into animal feedstuffs. As part of its package of control measures, the Ministry, with the cooperation of the feeding stuffs industry, introduced a number of voluntary codes of practice for the production, storage, handling and transport of animal feeding stuffs.

The Importation of Processed Animal Protein Order (HMSO, 1981b) prohibits the landing in Great Britain of any processed animal protein or any product containing processed animal protein except under the authority of a licence. The conditions imposed in the import licence reflect the likely contamination status of imported materials. In some countries, these conditions may require detention of every imported consignment at the port of landing until negative salmonella test results have been obtained.

DETECTION

Salmonella can be isolated from bacteraemic birds by direct culture but the caecum is the most likely site for isolation in adult birds for which selective enrichment is usually needed. However, the standard culture methods may lack sensitivity and more sensitive, rapid techniques have been developed to allow a greater throughput of samples (Davies and Wray, 1994c). Population screening methods must be capable of detecting low-incidence infections of poultry, which are common, and methods have been developed to sample the environment as an indirect indicator of flock infection.

Various isolation methods are in current use and most involve selective enrichment in selenite, tetrathionate or Rapport–Vassiliadis medium with incubation at 37–42°C and the use of selective plating media, such as MacConkey, deoxycholate citrate or brilliant green agar. Pre-enrichment in buffered peptone water, before selective enrichment in semisolid media such as Diassalm and plating on Rambach agar has been shown to be the most sensitive method.

Various serological tests are available for the detection of salmonella in poultry. The enzyme-linked immunosorbent assay (ELISA) is used in many countries for the identification of flocks infected with *S. enteritidis*, although bacteriological confirmation is recommended due to the lack of specificity. Two systems are in current use, the indirect ELISA and the competitive double antibody blocking ELISA, the former being favoured for monitoring purposes in Britain (WHO, 1994). One disadvantage of using a serological test is that positive results do not necessarily mean that the bird is still infected and negative results can be obtained in the early stages of infection prior to the development of an immune response. Interpretation of serological tests is further complicated by vaccination of flocks.

MONITORING FOR SALMONELLAS

Monitoring should be carried out at all stages of the production cycle. In breeding flocks and hatcheries it is mandatory, as indicated in the Breeding Flocks and Hatcheries Order (HMSO, 1993) if the breeding flock has more than 250 birds and the hatchery an incubator capacity of 1000 eggs or more. More intensive sampling can be carried out by checking fluff from the interior surfaces of the hatcher and broken eggshells from the trays. Surface swabs from different parts of the hatchery and sampling of macerated waste should assist in checking for the presence of salmonellas as well as the effectiveness of cleansing and disinfection.

At the rearing site, the presence of salmonellas in replacement birds can be checked by culturing of chick box-liners or swabs from the bottom of the boxes, chicks dead on arrival and those culled or dying within a few days of arrival. During the rearing period, bulked litter samples and dust from various sites, e.g. exhaust fans, provide the most convenient samples for monitoring. When breeders are in lay, the most reliable samples are nest-box floor swabs, nest-box litter, dust from internal feed hoppers and swabs from egg sorting tables and corridors; for elite birds more frequent sampling is desirable. Laying flocks may be sampled by using drag swabs in the manure pit, dust samples, swabs of the manure scraper and spilled debris from the egg collection belt. In the case of barn layers, litter, dust samples and nest boxes should be sampled.

After depopulation, and when cleansing and disinfection have been carried out, buildings should be checked for persistence of salmonellas. Samples should include large fabric swabs of earth floor surfaces or floor sweepings from concrete floors, nest-box floors, slave feed hoppers, beams, pipes and electrical fittings.

There is now evidence that the measures taken to eradicate *S. enteritidis* infection in the British poultry industry have had some success. Primary breeder flocks are free of infection and there is a declining trend in reports from parent breeding flocks (Fig. 11.3). However, eradication is still likely to be remote. Therefore, attention has also been directed at interventions to reduce the chance of infection or to eliminate infections that do occur. The most feasible are competitive exclusion with antibiotic treatment and vaccination. Competitive exclusion refers to colonization control in the live bird by the establishment of protective populations of intestinal bacteria (Nurmi and Rantala, 1973). Despite success under experimental conditions, this approach has shown mixed results in the field in its ability to protect against salmonella infection (Goren *et al.*, 1988; Mead, 1991; Mulder and Bolder, 1991). In general, protection is superior with undefined cultures that contain a broad range of bacteria (Stavric *et al.*, 1991), although there may be a risk of spreading pathogens to recipient birds. The use of antibiotic treatment is generally considered unwise due to the risk of selecting resistant strains of bacteria (particularly if quinolone drugs are used). Recent trials in British breeder flocks infected with *S. enteritidis* have shown that a combination of antibiotic treatment and competitive exclusion reduced the prevalence of infection but did not eliminate the organism (Reynolds *et al.*, 1997). Control by vaccination is

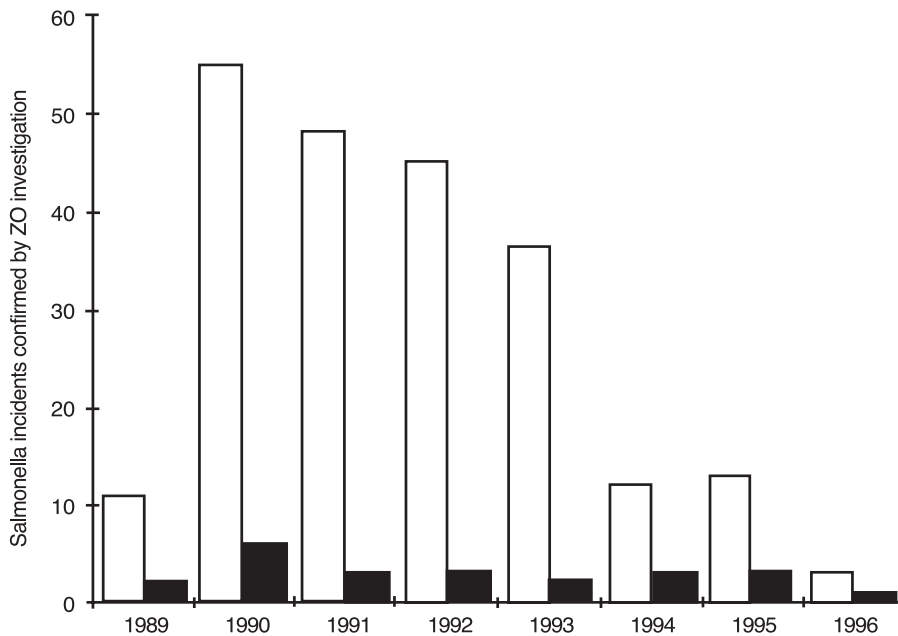


Fig. 11.3. Confirmed *Salmonella* incidents in broiler breeding flocks in Great Britain (1989–1996). ZO, Zoonoses Order 1989; □, *Salmonella enteritidis*; ■, *S. typhimurium*. Source: MAFF.

still in the developmental stages, although an inactivated vaccine is available in the UK and has been used successfully in breeding flocks. One disadvantage of vaccination is its interference with the results of serological monitoring. All three methods of intervention are likely to be most successful when used as part of a comprehensive salmonella control programme, with the main emphasis on a high level of disease security and hygiene.

CONCLUSIONS

Salmonella control presents a major challenge to all involved in animal production. During the *S. enteritidis* PT 4 epidemic, it became apparent that control must be based on a detailed knowledge of the epidemiology of infection and a specific control programme for each individual unit. An integrated programme is necessary covering all aspects of the supply chain from primary breeding flocks to the final product and it should include all those involved in poultry production. Many strategies were tried and tested e.g. slaughter, immunization, use of antibacterials. None was successful on its own, but improved hygiene and disease security, combined with vaccination has had the most impact on the *S. enteritidis* status of the UK poultry parent breeder sector and biosecurity measures alone have been effective in maintaining freedom from the organism in primary breeding flocks. There is a danger,

however, that vaccination for one particular serotype will create a niche for other serovars to emerge. Thus, future control measures must concentrate on eliminating or reducing all salmonella serotypes by comprehensive disease security precautions assisted, perhaps, by the use of multivalent salmonella vaccines in specific cases.

THE FUTURE

As the prevalence of human salmonellosis continues to increase, there is recognition that a 'farm to fork' approach is necessary to reduce pathogens, and Good Manufacturing Practices (GMPs) using Hazard Analysis Critical Control Point (HACCP) principles are being increasingly used to effect a reduction. Using the HACCP system applied to the critical control points on farms and supplies of feed and services will be beneficial in future. As we have shown earlier, the prevalence of salmonella in the hatchery can be reduced by a comprehensive planning and sanitization programme. On the farm, it is necessary to combine effective biosecurity with monitoring and our studies have shown (Davies and Wray, 1996b) that monitoring of the litter and environment is more reliable than sampling individual birds. It is important that the critical control points are identified for each individual farm and that the application of HACCP is maintained by key-point process monitoring. There are, however, still many unanswered questions on the most effective and economic means of controlling *Salmonella* in the poultry industry. This is an area where more investigative bacteriology, using the principles of Best Practice Analysis, is required to identify existing effective measures (and errors) and to disseminate information on effective techniques.

REFERENCES

- Allen, P.C. (1993) Effects of formaldehyde fumigation of housing on carotenoid pigmentation in three breeds of chickens. *Poultry Science* 72, 1040–1045.
- Angen, O., Skov, M.N., Chriel, M., Agger, J.F. and Bisgaard, M. (1996) A retrospective study of *Salmonella* infections in Danish broiler flocks. *Preventive Veterinary Medicine* 26, 223–237.
- Arakawa, A., Fukaton, T., Baba, E., McDougald, L.R., Bailey, J.S. and Blankenship, L.C. (1992) Influence of coccidiosis on colonisation in broiler chickens under floor pen conditions. *Poultry Science* 71, 59–63.
- Baggesen, D.C., Olsen, J.E. and Bisgaard, M. (1992) Plasmid profiles and phage types of *Salmonella typhimurium* isolated from successive flocks of chickens on three parent farms. *Avian Pathology* 21, 569–579.
- Bailey, J.S. (1993) Control of *Salmonella* and *Campylobacter* in poultry production, a summary of work at Russell Research Centre. *Poultry Science* 72, 1169–1173.
- Bailey, J.S., Cox, N.A. and Berreang, M.E. (1994) Hatchery acquired *Salmonellae* in broiler chicks. *Poultry Science* 73, 1153–1157.

- Bale, J., Bennett, P.M., Beringer, J.E. and Hinton, M.H. (1993) The survival of bacteria exposed to desiccation on surfaces associated with farm buildings. *Journal of Applied Bacteriology* 75, 519–528.
- Brown, D.J., Olsen, J.E. and Bisgaard, M. (1992) *Salmonella enteritidis* infection, cross-infection and persistence within the environment of a broiler parent stock unit in Denmark. *Zentralblatt für Bakteriologie* 277, 129–138.
- Bruce, J. and Drysdale, E.M. (1990) Egg hygiene: routes of infection In: Tullett, S.G. (ed.) *Avian Incubation*. Butterworth, London, pp. 257–267.
- Cason, J.A., Cox, N.A. and Bailey, J.S. (1991) Survival of *Salmonella typhimurium* during incubation and hatching of inoculated eggs. *Poultry Science* 70 (Suppl. 1), 152.
- Corrier, D.E., Hargis, B.M., Hinton, A. and Deloach, J.R. (1993) Protective effect of used poultry litter and lactose in the feed ration on *Salmonella enteritidis* colonisation of Leghorn chicks and hens. *Avian Diseases* 37, 47–52.
- Davies, R.H. and Wray, C. (1994a) *Salmonella* pollution in poultry units and associated enterprises. In: Dewi, I.A.P., Axford, R.F.F., Marai, I.F.M. and Umed, H. (eds) *Pollution in Livestock Systems*. CAB International, Wallingford, pp. 137–166.
- Davies, R.H. and Wray, C. (1994b) Use of larvae of *Lucilia serricata* in colonisation and invasion studies of *Salmonella enteritidis* infection in poultry Flair No 610. In: Pusztai, A., Hinton, M.H. and Mulder, R.W.A.W. (eds) *The Attachment of Bacteria to the Gut*. COVP-DLO, Het Spelderholt, pp. 117–123.
- Davies, R.H. and Wray, C. (1994c) An approach to reduction of *Salmonella* infection in broiler chicken flocks through intensive sampling and identification of cross-contamination hazards in commercial hatcheries. *International Journal of Food Microbiology* 24, 147–160.
- Davies, R.H. and Wray, C. (1994d) Evaluation of a rapid cultural method for identification of *Salmonellas* in naturally contaminated veterinary samples. *Journal of Applied Bacteriology* 77, 237–241.
- Davies, R.H. and Wray, C. (1995a) Observations on disinfection regimens used on *Salmonella enteritidis* infected poultry units. *Poultry Science* 74, 638–647.
- Davies, R.H. and Wray, C. (1995b) Mice as carriers of *Salmonella enteritidis* on persistently infected poultry units. *Veterinary Record* 137, 337–342.
- Davies, R.H. and Wray, C. (1996a) Persistence of *Salmonella enteritidis* in poultry units and poultry food. *British Poultry Science* 37, 589–596.
- Davies, R.H. and Wray, C. (1996b) Determination of an effective sampling regime to detect *Salmonella enteritidis* in the environment of poultry units. *Veterinary Microbiology* 50, 117–127.
- Edel, W., van Schothorst, M., Guinee P.A.M. and Kampelmacher, E.H. (1973) Mechanisms and prevention of *Salmonella* infection in animals In: Hobbs, B.C. and Christian, J.H.B. (eds) *The Microbiological Safety of Food*. Academic Press, London, pp. 247–256.
- Fris, C. and van den Bos, J. (1995) A retrospective case control study of risk factors associated with *Salmonella enteritidis* infection on Dutch broiler farms. *Avian Pathology* 24, 255–272.
- Geissler von, H and Kösters, J. (1972) Die hygienische Bedeutung des Getreidesehim melkäfers (*Alphitobius diaperinus* Panz) in der Geflügelmast. *Deutsche Tierärztliche Wochenschrift* 79, 177–181.
- Goren, E., de Jong, W.A., Doornenbal, P., Bolder, N.M., Mulder, R.W.A.W. and Jansen, A. (1988) Reduction of *Salmonella* infection of broilers by spray application of intestinal microflora: a longitudinal study. *Veterinary Quarterly* 10, 249–255.
- Haigh, T. and Betts, W.B. (1991) Microbial barrier properties of hen egg shells. *Microbios* 68, 137–146.

- Hammack, T.S., Sherrod, P.S., Bruce, V.R., June, G.A., Satchell, F.B. and Andrews, W.H. (1993) Growth of *Salmonella enteritidis* in grade A eggs during prolonged storage. *Poultry Science* 72, 373–377.
- Henzler, D.J. and Opitz, H.M. (1992) The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. *Avian Diseases* 36, 625–631.
- HMSO (1981a) The Animal Health Act 1981. *Statutory Instrument 1975* No. 1030.
- HMSO (1981b) Importation of Processed Animal Protein Order. *Statutory Instrument* No. 677.
- HMSO (1989a) The Zoonoses Order 1989. *Statutory Instrument 1989* No. 285.
- HMSO (1989b) The Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989. *Statutory Instrument 1989* No. 1963.
- HMSO (1989c) The Processed Animal Protein Order 1989. *Statutory Instrument 1989* No. 661.
- HMSO (1993) The Poultry Breeding Flocks and Hatcheries Order 1993. *Statutory Instrument 1993* No. 1898.
- Holt, P.S. (1993) Effect of induced molting on the susceptibility of white leghorn hens to a *Salmonella enteritidis* infection. *Avian Diseases* 37, 412–417.
- Humphrey, T.J., Baskerville, A., Chart, H., Rowe, B. and Whitehead, A. (1992) Infection of laying hens with *Salmonella enteritidis* PT4 by conjunctival challenge. *Veterinary Record* 131, 386–388.
- Krabisch, P. and Dorn, P. (1980) Zur epidemiologischen Bedeutung von Lebendvektoren bei der Verbreitung von Salmonellen in der Geflügelmast. *Berliner and Munchener Tierärztliche Wochenschrift* 92, 232–235.
- Kradel, D.C. and Miller, W.L. (1991) *Salmonella enteritidis* observations on field related problems. In: *Proceedings of the Fortieth Western Poultry Disease Conference*, Acapulco, pp. 146–150.
- Lister, S.A. (1988) *Salmonella enteritidis* infection in broilers and broiler breeders. *Veterinary Record* 123, 350.
- Mario-Padron, N. (1990) *Salmonella typhimurium* penetration through the egg-shell of hatching eggs. *Avian Diseases* 34, 463–465.
- Mayer, F.J. and Takeballi, M.A. (1983) Microbial contamination of the hen's egg: a review. *Journal of Food Protection* 46, 1092–1098.
- Mead, G.C. (1991) Developments in competitive exclusion to control *Salmonella* carriage in poultry. In: Blankenship, L.C. (ed.) *Colonization of Human Bacterial Enteropathogens in Poultry*. Academic Press, New York, pp. 91–104.
- Milner, K.C. and Shaffer, M.F. (1952) Bacteriologic studies of experimental *Salmonella* infections in chicks. *Journal of Infectious Diseases* 90, 81–85.
- Mulder, R.W.A.W. and Bolder, N.M. (1991) Experience with competitive exclusion in the Netherlands. In: Blankenship, L.C. (ed.) *Colonization of Human Bacterial Enteropathogens in Poultry*. Academic Press, New York, pp. 77–90.
- Nakamura, M., Nagamine, N., Takahashi, T., Norimatsu, M., Suzuki, S. and Sato, S. (1995) Intratracheal infection of chickens with *Salmonella enteritidis* and the effect of feed and water deprivation. *Avian Diseases* 39, 853–858.
- Nascimento, V.P. and Solomon, S.E. (1991) The transfer of bacteria (*Salmonella enteritidis*) across the eggshell wall of eggs classified as 'poor quality'. *Animal Technology* 42, 157–165.
- Nascimento, V.P., Cranstown, S. and Solomon, S.E. (1992) Relationship between shell structure and movement of *Salmonella enteritidis* across the egg shell wall. *British Poultry Science* 33, 37–48.
- Nurmi, E. and Rantala, M. (1973) New aspects of *Salmonella* infection in broiler production. *Nature* 241, 210–211.

- O'Brien, J.D.P. (1988) *Salmonella enteritidis* infection in broiler chickens. *Veterinary Record* 122, 214.
- Opitz, H.M. (1992) Progress being made in *Salmonella enteritidis* reduction on the farm. *Poultry Digest* March, 16–22.
- Reynolds, D.J., Davies, R.H., Richards, M. and Wray, C. (1997) Evaluation of combined antibiotic and competitive exclusion in broiler breeder flocks infected with *Salmonella enterica* serovar enteritidis. *Avian Pathology* 26, 83–95.
- Stavric, S., Gleeson, T.M. and Blanchfield, B. (1991) Efficacy of undefined bacterial treatments in competitive exclusion of *Salmonella* from chicks. In: Blankenship, L.C. (ed.) *Colonization of Human Bacterial Enteropathogens in Poultry*. Academic Press, New York, pp. 323–330.
- Turnbull, P.C.B. (1979) Food poisoning with special reference to *Salmonella* – its epidemiology, pathogenesis and control. *Clinical Gastroenterology* 8, 663–714.
- Watson, W.A. and Kirby, F.D. (1984) The *Salmonella* problem and its control in Great Britain. In: *Proceedings of the International Symposium on Salmonella*. American Association of Avian Pathologists, Kennett Square, USA, pp. 35–47.
- WHO (1994) Wray, C. and Davies, R.H. (eds) *Guidelines on Detection and Monitoring of Salmonella Infected Poultry Flocks with Particular Reference to Salmonella enteritidis*. WHOZoon/94/173.

