The paratyphoid salmonellae

P.A. Barrow
Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN, United Kingdom

Summary

The paratyphoid Salmonella bacteria, comprising more than 2,000 serovars or serotypes, are a major problem to the poultry industry. This is largely the result of entry of the bacteria into the human food chain. Human infection takes the form of a gastroenteritis, which in highly susceptible individuals can result in death. Some strains of certain serotypes have the capacity to produce morbidity and mortality in very young chickens. In adult birds, some serovars become localised in the reproductive tract with concomitant vertical transmission. The association between S. Enteritidis and table eggs has produced a great deal of publicity and concern with resulting national and international attempts at controlling the major serotypes, S. Typhimurium and S. Enteritidis, at the breeder and layer level. Control is possible through rigorous hygiene and management procedures, but is not always economically viable. As a result, control by serological and bacteriological testing and slaughter may also not be feasible. Antibiotics have been used to reduce carriage but can cause problems of resistance and increased susceptibility. The use of growth promoting antibiotics can also increase susceptibility to infection. Other means of increasing the resistance of poultry to infection are therefore being sought. These include the use of intestinal flora preparations for competitive exclusion of Salmonella from newly hatched chicks. Killed and live vaccines are used, both with some success. However, the safety of some live vaccines is still uncertain.

Keywords

Description of the disease in poultry

The capacity of strains of Salmonella enterica to produce disease in poultry is related to serotype. Thus, the serotypes Typhimurium and Enteritidis are well known to produce clinical salmonellosis in poultry (152) but, less frequently, other serotypes such as Montevideo, Thompson and others may also be implicated (50).

Mortality rates can vary enormously, normally from less than 10% to over 80% in severe outbreaks (129, 152). This is true for both chicks and poults (41, 120). Under experimental conditions, mortality and morbidity are also strain dependent. Even within serotype, strain virulence can vary widely from 0% to almost 100%. In the field, these figures are never reached, probably because of the rapid rise in resistance observed during the first 24 h to 48 h of life and the fact that the levels of infection are never 100% within the first 24 h. The rise in resistance to infection (148) is attributed to maturation of macrophages, although the significance of this in terms of cellular changes is not known. Experimental infection of birds over this age results in little or no clinical disease but considerable intestinal colonisation, depending to some extent on the genetic background of the bird.

Transmission of infection is generally considered to occur by mouth. Massive bacterial multiplication occurs within the gut and tissue invasion occurs rapidly (27). The course of the disease is then one of multiplication within the reticuloendothelial system and progressive malaise. The clinical signs are anorexia and adipsia, depression with ruffled feathers and increasing drowsiness. Signs of enteritis may be present, indicated by accumulations of faecal material around the vent. Death generally follows four to ten days after infection. Convalescent birds will show different degrees of stunting for several weeks afterwards.
Post-mortem examination may reveal a persistent yolk sac that may be heavily infected with Salmonella. One of the characteristics of infection caused by the recent strains of S. Enteritidis has been a polyserositis. Paruline pericarditis may be evident (20, 58). Small areas of necrosis may be present on the surface of the liver and spleen. Hepatomegaly and splenomegaly may also be observed. Caseous cores of caseous inflammatory exudate are also common.

As with field outbreaks of fowl typhoid, the genetic background of the host may be important in the incidence of clinical disease. Under experimental conditions, mortality produced by a virulent strain may vary from 25% to 100% between different inbred lines (27, 48). Similar differences have also been found between different commercial lines of layers and broilers (88), and heterogeneity of susceptibility may also be found in highly outbred lines (20).

**Cost to the poultry industry**

Estimates of the cost of salmonellosis (paratyphoid) to the poultry industry are very difficult to obtain. Damage may be direct, because of losses to newly hatched chicks due to clinical infection. However, these events are relatively rare and are not economically significant. Indirect effects may result from the public health impact of poultry-derived human infection. This can be divided into costs associated with the treatment of human infection and losses of earnings and productivity. National costs associated with eradication have been borne by several governments, including those of the United Kingdom (UK), the Netherlands and Sweden. These include the cost of insurance (175) and the cost of replacement and compensation. The cost of paratyphoid in poultry to the United States of America (USA) was estimated to be US$77 million per annum in 1976 (111), while Roberts and Sockey have estimated the medical costs and lost productivity from food-borne salmonellosis to be US$553 million to US$988 million annually in the USA (140). This may not be totally poultry derived, but a considerable proportion will be so. Losses also include the fall in sales due to the adverse publicity associated with large outbreaks, as occurred in the UK in the late 1980s.

**Description of the agent**

**Taxonomy**

The taxonomy of the genus Salmonella has been, at times, chaotic and confusing and remains so to this day. On the basis of deoxyribonucleic acid (DNA-DNA) hybridisation studies, the 2,000 serotypes (previously erroneously regarded taxonomically as species) are now recognised as belonging to two species, S. enterica and S. hongori. The S. enterica species is divided into several subspecies, including S. enterica subsp. enterica, salamae, arizonae, diarizonae, houtenae and indica. Beyond this, S. enterica subsp. enterica is then subdivided into serotypes (or serovars), some of which are well-known. As these are not regarded as species, the serotype name is not written in italics and should be presented, for example, as S. enterica ser. Typhimurium or S. Typhimurium. However, this scheme (107) will take some time to be fully accepted and users of the old italicised binomial system will not be ostracised.

From the practical point of view of both animal and public health bacteriologists, the precise presentation of the name is not a problem, since whether a serotype is known as S. typhimurium, S. Typhimurium or S. enterica subsp. Typhimurium, the serotype being discussed will be clear. Some confusion remains with the current designation of the serotypes S. Gallinarum and S. Pullorum, but this remains beyond the scope of this chapter.

**Bacteriology**

Salmonella is a member of the Enterobacteriaceae. These organisms are Gram negative, non-sporing rods which used to belong to the genus Bacterium. Most strains of paratyphoid are motile by means of peritrichous flagella. A number of strains produce type 1 (common) pili which are present in large numbers around the cell. A high proportion of strains that produce systemic disease are unable to produce type 1 pili (70). A number of other pilus types are also produced which are thought to be associated with virulence (25), although in some cases, such as SEF-14 in S. Enteritidis, this has been difficult to demonstrate (160). The bacteria produce butyrous colonies on ordinary nutrient agar media and grow optimally at 37°C. Most strains are identical in appearance on selective media such as brilliant green agar. Closely related micro-organisms such as Citrobacter may have a very similar appearance on such agar and given the antigenic relatedness of the two, such bacteria may occasionally cause confusion during identification procedures. Many strains grow well at 43°C (closer to the body temperature of the chicken) and selective broth enrichment with selenite or Rappaport-Vassiliadis (RV) medium utilises this property. Samples can be stored for considerable periods in stab nutrient agar cultures or on Dorset's egg medium kept at 4°C. The bacteria show normal sensitivity to heat and chemical agents (98).

Salmonella are facultatively anaerobic. Most serotypes are prototrophic, although 7% of S. Typhimurium strains require cysteine and nicotinamide. Considerable variation in biochemical activities can be observed. Most serotypes produce acid and gas from glucose, mannitol and sorbitol. Many serotypes utilise citrate as a sole carbon source and produce hydrogen sulphide. Lysine and ornithine decarboxylase are also produced. Serotypes generally do not ferment sucrose, salicin or adonitol and are urease and indole negative and do not deaminate phenylalanine. They are methyl-red positive and Voges-Proskauer negative. Apart from these last two reactions, occasional strains differ in their reactions to many of the above.
Antigenic structure

The major components of the bacterial surface used in epidemiological investigations are the flagella (designated H antigens) and lipopolysaccharide (LPS) (designated O antigens). Many of the antigenic epitopes are shared with other genera of the Enterobacteriaceae and cross-reactions may be common with closely related taxa such as Citrobacter. A detailed description of the fine structure of LPS or other surface antigens may be found in standard text books (132).

The O antigens are the epitopes on repeated hydrophilic linear or branched sub-units of carbohydrate. They may be recognised by agglutination with absorbed antiserum. Single epitopes or more than one may be present and the epitopes are shared between many different serotypes. The epitopes are not constant for any serotype and may change by acquisition of extraneous genes from other Salmonella or Citrobacter strains by transduction (phage mediated) or conjugation (plasmid). As O antigens also act as receptors for a variety of bacteriophages, Salmonella strains can become resistant to phage activity by mutation in the LPS genes and loss of the O antigen.

Flagellar (H) antigens are also possessed by many serotypes. Two flagella antigen genes are present, only one of which is expressed at any one time (phase 1 or phase 2). This is regulated by the spontaneous inversion of a DNA segment that controls expression. Several epitopes may be associated with expression of either gene.

Molecular biology

Genome and gene structure and its relationship to virulence and disease production is a rapidly developing area of research. Further information may be obtained elsewhere (106).

Serotypes such as S. Typhimurium, S. Enteritidis and strains of some other serotypes are capable of producing a typhoid-like disease in very young chicks and poults. These strains contain a large plasmid encoding a number of virulence genes including a cluster (spy genes) whose exact function is unknown but which is essential for the production of systemic disease. These genes also encode for a number of pil-like structures, some of which are required for the initial intestinal phase of infection (although these serotypes do not easily colonise the intestine) but the majority of which have unknown functions. The plasmid of the wild-type strains tested is not self-transmissible and has not been transferred by prophages possessed by many of the strains. The virulence plasmid alone is insufficient to produce disease since transfer to a laboratory Escherichia coli K 12 strain does not result in an increase in virulence.

The chromosome of S. enterica contains a number of virulence gene clusters, the so-called Salmonella pathogenicity islands (SPI). These have different functions although their complete roles in virulence are still being studied. The SPI-1 encodes a type III secretion system involving the inv/spa genes required for invasion. The SPI-2 encodes a second type III secretion system, genes of which are induced under low pH and intracellularly. The SPI-3 and SPI-4 are poorly understood, but the former contains some regulators of genes required for survival in macrophages and the latter has been identified by transposon mutation. The SPI-5 is required for the production of enteritis and fluid secretion in intestinal loops and may therefore be responsible, in part at least, for diarrhoea production. Additional individual genes, such as sopA and sopE, which are required for fluid secretion, are found at additional sites on the chromosome, some of which are flanked by phage-like elements. In the case of sopE, the phage itself has been induced by rescuing and enabling transduction of the sopE gene.

Some of the genes associated with colonisation of the intestines of chickens have been identified (61, 167), and these indicate that for much of the time Salmonella organisms are under nutritional and other stress conditions in the alimentary tract of the chicken and may not be ideally adapted to that environment. Indeed, S. Typhimurium and S. Enteritidis may originally have been murine strains, and many other serotypes may originate in reptiles. The entry of these strains into and persistence in poultry may be accidental and reflect the closely integrated nature of much commercial poultry production.

Antibiotic resistance

Antibiotic usage and regulation in the poultry industry varies considerably world-wide. This extends from the administration by veterinarians in some countries, such as the UK, to unrestricted purchase and administration by farmers in many countries where the poultry industry is less intense. Antibiotics which are effective against Salmonella may be used to control clinical disease in young chickens. In addition, antibiotics have also been advocated for controlling carriage by laying hens. However, in the absence of clinical salmonellosis, Salmonella is subject to contact with antibiotics when they are used against other bacterial disease such as infection with E. coli. Given this situation, the relatively small amount of resistance to the major chemotherapeutic agents is surprising, when compared with the calf rearing industry in a number of countries. In general, multi-resistant strains which are found in poultry (162) are thought to originate in cattle (mainly S. Typhimurium, but in some countries such as the USA, other serotypes are also implicated). Where major outbreaks of infection occur in humans, these are generally regarded to arise principally from the consumption of poultry meat. These strains are generally sensitive to most antibiotics.

A number of strains of S. Enteritidis phage type 4 have been found to have increased resistance levels to furazolidone. This reflects use of this drug in the poultry industry. Similarly, the massive increases in the use of quinolones and fluoroquinolones since the mid-1980s has led to the isolation
of an increasing number of Salmonella strains which are resistant to nalidixic acid (135, 186). Many of the strains isolated in the UK are thought to originate elsewhere but this is not true for all strains. The incidence of quinolone resistance in Campylobacter jejuni has also increased over a similar period of time and to a much greater degree (163).

Typing

Serotyping

As indicated, the genus Salmonella may be divided biochemically, although this has limited practical use in terms of epidemiology. A scheme (Kauffmann-White) based on the O and H antigens of Salmonella has been developed and refined over many years and remains the standard for typing individual strains. The combination of O and H antigen complement determines the Salmonella serotype. Sets of antisera are used to identify the specific O and H antigens possessed by strains of interest. Identification is by agglutination. As indicated above, many O and H antigens are shared by related genera within the Enterobacteriaceae which may cause some minor practical problems.

Serotypes possessing the same major O antigens are grouped together. Thus, group B (including S. Typhimurium and many others) possess the 4-O antigen and S. Enteritidis is classed in group D which possess the 9-O antigen. Rough strains, which do not react specifically, exist in the wild and are designated rough. They are non-typable and agglutinate in the presence of acriflavine and a variety of unrelated antisera. Very rough strains may agglutinate spontaneously in saline.

Identification of H antigens may require both phases to be identified. This may require incubation in the presence of antiseraum against the expressed or dominant phase to allow selection of the small number of bacterial cells in the culture expressing the minority antigen. The full Kauffmann-White scheme for serological identification is described in Ewing (74) and technical details in Ørskov and Ørskov (131).

Phage typing

Serotypes such as S. Typhimurium and S. Enteritidis have predominated in the poultry industry and human illness for many years and currently the isolation of either of these serotypes from an outbreak of human infection, poultry meat and a suspect farm may not be sufficient evidence of a causal relationship. Further subdivision is possible, based on the susceptibility of the strain in question to a set of bacteriophages (89). The susceptibility may be dependent on the following factors:

a) whether the receptor for the phage is possessed by the bacterium, as occurs with S. Typhi

b) whether a previous infection has resulted in incorporation of the phage DNA into the chromosome (lysogeny), rather than resulting in virulent, lytic infection.

A degree of standardisation is attempted world-wide within the scheme in use, such that phage type identity can be assured with identical strains tested in different laboratories. In cattle infections particularly, this method has been essential in following the course of infection and strain evolution as a result of pressure from antibiotic usage.

Biotyping

The pattern of biochemical reactions performed in standard assays may be used to sub-divide serotypes or phage types. This system has been used with S. Typhimurium and other serotypes (130).

Plasmid typing and other deoxyribonucleic acid methods

The plasmid complement of individual strains may be analysed by separation by gel electrophoresis. This is a cheap and simple method, but has not been used as extensively as hoped. Profiles for unrelated strains may be very similar and further division may require digestion of the plasmid DNA by restriction nucleases and separation of the digestion fragments by electrophoresis. Digestion of chromosomal DNA may also be used, either by restriction enzymes which yield many fragments which may be analysed by computer restriction enzyme analysis or by enzymes which cut rarely and yield a small number of fragments which must be separated carefully by pulsed field gel electrophoresis. Neither of these methods has been taken up extensively, although the appearance of S. Enteritidis phage type 4 in many countries has required a number of these techniques to be used simultaneously for epidemiological investigations. A combination of DNA-DNA hybridisation and restriction analysis using probes against ribosomal ribonucleic acid genes or IS200 may also be used for strain discrimination. More recently, polymerase chain reaction (PCR)-based techniques have been developed in which random amplification of DNA fragments using artificially selected primers not based on real sequence information has been used (random amplified polymorphic DNA typing). A similar technique using amplification of ribosomal DNA (rDNA) followed by digestion has also been developed.

These techniques are currently being refined and the extent to which they will contribute in a practical way to epidemiology remains to be seen. An introduction to these methods has been presented by Mendoza and Landeras (115).

Pathogenicity in poultry

As indicated above, strains of paratyphoid Salmonella produce a wide variety of infections in poultry. This depends on bacterial serotype and strain, age of the bird, genetic background of the host and portal of entry.

Unlike the 'typhoid' serotypes such as S. Typhi in humans and S. Gallinarum in chickens, paratyphoid serotypes are unable
to produce severe systemic disease in physiologically and immunologically normal, healthy, adult chickens. Clinical disease is normally only produced in chickens or turkey poults when the birds are infected within a few hours of hatching. Of the over 2,000 paratyphoid serotypes identified, the virulence for poultry of only a few has been determined, although many more have been observed in the field to produce systemic salmonellosis in chicks (122). Some have also been tested experimentally. Strains of *Salmonella Typhimurium* vary considerably in capacity to produce systemic disease in newly hatched chicks, with mortality ranging from 0% to 100%. Variation in virulence was observed by both oral and systemic routes of administration but the capacity to invade from the alimentary tract was deemed to be the most important virulence characteristic. Strains of *S. Enteritidis* also vary considerably, although fewer have been tested (21). Other serotypes appear to be less virulent (148).

Infection of newly hatched chicks by the oral route is quickly followed by massive multiplication of bacteria in the gut where very high numbers are reached and thus, extensive shedding in the faeces can occur. Association with the gut wall followed by invasion occurs rapidly, and bacteria are taken to the reticulo-endothelial system where multiplication occurs, probably primarily intracellularly, if bacterial and host genetics are appropriate. This may eventually result in death. Whether enteritis occurs is unclear. Some disruption of intestinal function can be observed, which is characterised by accumulations of food and fluid around the vent indicating gut dysfunction. This is not observed after parenteral administration of the bacteria.

The virulence plasmid of neither *S. Typhimurium* nor *S. Enteritidis* is required for virulence in the young chick, unlike the situation with typhoid infections produced by these serotypes in adult mice or in *S. Gallinarum* infection in adult birds. Invasion genes (the inv/spa genes of SPI-1) are important, at least in the initial invasion stage.

Considerable variation occurs in the severity of the disease produced, depending on the genetic lineage of the bird. Bumstead and Barrow found that the variation in mortality produced by oral inoculation of a virulent strain of *S. Typhimurium* was extreme and occurred whether the bacteria were inoculated orally or parenterally, thus indicating expression of this characteristic at the level of the reticuloendothelial system (48). The same lines were resistant/susceptible to *S. Enteritidis*, *S. Gallinarum* and *S. Pullorum* (49). The effect was neither sex-linked nor associated with the major histocompatibility complex. The trait was inherited in a Mendelian fashion and is likely to be the result of a single gene, now nominated *Sal*. Some differences have also been found in the patterns of intestinal colonisation in different commercial lines of chickens (69), but the exact nature of this difference has yet to be studied in detail.

Infection of chickens of more than a few days old with a virulent paratyphoid *Salmonella* strain generally results in little or no clinical disease. As a result of the presence of a complex intestinal flora, the multiplication of the *Salmonella* is less extensive. Nevertheless, invasion occurs and bacteria reach the liver and spleen, but as the macrophages are more inhibitory, no intracellular multiplication occurs and the *Salmonella* organisms are gradually killed.

In hens which are in lay, some localisation of the invaded organisms occurs in the reproductive tract, particularly the ovary and oviduct (102). This can occur with several serotypes, but has been reported most recently with certain phage types of *S. Enteritidis*. The manner in which the organism localises in these tissues is not fully known but some evidence demonstrates the ability of this organism to invade the granulosa cells which occur in a layer surrounding the pre-ovulatory follicle (157). Other work shows evidence of infiltration of the oviduct by macrophages at the onset of sexual maturity (37). This may be important in allowing spread of infection (of *S. Enteritidis* and *S. Pullorum*) from the spleen to the oviduct at this time. This results in egg transmission by these serotypes, with associated public health consequences and vertical spread of infection to progeny. Egg contamination may also occur by faecal surface contamination of the shell at the time of lay (30). Shell penetration of freshly laid eggs by *Salmonella* has been demonstrated (47, 100).

'Paratyphoid' serotypes of *Salmonella* are not host-adapted or host-specific, unlike serotypes such as *S. Typhi* and *S. Gallinarum*. *Salmonella Typhimurium* and *S. Enteritidis* have the capacity to produce typical typhoid infections in adult mice, but as noted above, generally do not do so in adult poultry. Indeed, the host specificity of 'typhoid' *Salmonella* serotypes may be regarded as being either mammalian or avian specific. Although *S. Dublin* and *S. Choleraesuis* produce typical disease in cattle and pigs respectively, they also produce typical disease in mice, and in the case of *S. Choleraesuis*, in many other mammals. In contrast, *S. Gallinarum* and *S. Pullorum* produce typhoid disease in birds only (34). Past literature suggests that many of these serotypes may also produce gastroenteritis, although detailed studies on these infections have not been undertaken.

Most of the above studies have been performed on chickens, but it seems likely that turkeys behave similarly. Some evidence exists of paratyphoid systemic infections in poults (122). In ducks there is little such evidence. A recent study showed that a commercial line of ducks in the UK was very resistant to systemic disease, even by *S. Gallinarum* (36). This resistance was expressed at the level of the reticuloendothelial system. The potential of ducks to lay infected eggs has been recognised for many years (143).
Although adult birds are relatively resistant to systemic multiplication by paratyphoid Salmonella serotypes, these serotypes nevertheless colonise the alimentary tract in the absence of disease. This results in carcass contamination and entry into the human food chain. Experimental oral infection of day-old chicks results in faecal excretion of large numbers of Salmonella bacteria for many weeks. Infection of adult birds results in much reduced excretion of Salmonella. The difference is that the adult has acquired a complex gut flora which is inhibitory to colonisation by pathogens. This has been exploited commercially (see below). The main sites of localisation are the caeca, and to a lesser extent, the crop. This is probably the result of a slower rate of flow of contents through these organs. No evidence exists for specific attachment to the mucosa in large numbers as a prerequisite for colonisation. Indeed, evidence suggests that this does not occur except as an initial invasion stage in systemic disease. Serotypes such as S. Choleraesuis, S. Gallinarum and S. Pullorum do not colonise, except as a consequence of clinical disease (S. Gallinarum only).

**Epidemiology**

Unlike the 'typhoid' serotypes, the epidemiology of the 'paratyphoid' serotypes is complex. This is a result of the fact that these serotypes are not host-specific and are shed in relatively large numbers from the alimentary tract without signs of disease. The sources of infection for poultry are many, although the major sources can be limited to poultry themselves, feed and the environment. This has been endorsed several times by the World Health Organization (WHO) and other organisations and symposia (5, 10, 11, 179, 182).

**Poultry as a source of infection**

Reference has already been made to vertical transmission in the production of eggs, which leads to infected chicks. The exact course of events during the incubation of infected eggs is unknown, but immediately after hatching, oral ingestion by the chicks results in very high numbers of Salmonella in the gut and extensive shedding in the faeces. The capacity for horizontal spread as a result of hatchery infection is thus very high. Ingestion of contaminated fluff, eggshell or other dust may result in infection in the incubator. This can result in extensive contamination of the hatchery which may continue for a long time. Infection of this sort can last the life of a broiler, and extensive cross-contamination of other birds in the same house can occur. Replacement of birds may also introduce new infections, and transfer from house to house on the same site may also occur. This is particularly true of sites where birds of several ages co-exist.

The duration of faecal excretion may be affected by several external factors, such as external temperature, use of antibiotics and growth promoters and concomitant infection by other agents such as Eimeria (76) and infectious bursal disease (134), both of which may exacerbate Salmonella infection.

**Feed as a source of infection**

Feed has been recognised as a source of infection for many years and the subject has been comprehensively reviewed (177). One of the major sources of infection for poultry feed is the animal protein component. Traditionally, dried fish meal has been an important protein component and the method of air drying by the sun in the countries of production leads inevitably to contamination by faecal material from wild birds and reptiles. However, proteins from other sources may also be contaminated (149), and the carbohydrate and even mineral components may also become contaminated. However, the use of Salmonella-free feed materials does not preclude infection, since this can occur at the time of milling or afterwards by inappropriate storing which results in contamination by rodents.

**Environment as a source of infection**

Many sources of infection for poultry are present in the environment. These may be a result of the housing itself, rodents, wild birds and cats which may have access to the housing, water sources and animal attendants who may introduce infection.

In discussing housing, the fact that poultry rearing occurs in many different climates must be considered. In temperate climates, the birds may be totally enclosed and restricting access by vermin may be easier to achieve. In hotter climates, this may be very difficult as open-sided housing is common to enhance ventilation.

Rodents have long been recognised as sources of infection (either by introducing new strains or by becoming infected from stock in a house and passing that infection to the next batch of birds in the house) (96). Any other mammals which are kept close to a poultry house must also be regarded as potential sources of infection. Domestic cats are sometimes kept to reduce rodent numbers on site and these may also act as reservoirs of infection.

Personnel may transfer Salmonella organisms from house to house or between sites on clothing, particularly on footwear.

The introduction of infection can lead to rapid spread within a flock and resulting heavy contamination of the house. After depopulation, the contaminated house may be difficult to clean, depending on the type of construction and age, which may lead to contamination of the next batch of birds.

**Transmission to humans**

Faecal shedding may result in contamination of feathers and skin. During depopulation and transport to slaughter premises, stress can lead to increased shedding and close proximity of other birds in crates can result in extensive cross-contamination. Crates themselves can contaminate birds...
during transport. Levels of infection of less than 5% in a flock can lead to carcass contamination levels of 50% to 100% in retail outlets.

The finer points of slaughterhouse technique and the numerous factors that can lead to further cross-contamination between carcasses have been described elsewhere (112), and will not be detailed here. However, scalding tanks can become contaminated with faeces, plucking machines can spread infection, intestinal rupture can occur during evisceration and pooling giblets (gizzard, heart, neck, etc.) can lead to infection being reintroduced to previously uninfected carcasses. Considerable effort is made to reduce spread by chemical treatment and water additives, but the economics of slaughter dictate that only limited measures can be taken and that the most sound approach is to ensure that batches of birds are free of infection before slaughter or that Salmonella-free birds are slaughtered before infected batches.

Pooling material (e.g. minced meat and liquid egg) inevitably results in greater frequencies of infection.

Table eggs are also now considered to be possible sources of infection. The levels of infection are low and generally much below the incidence of contaminated carcasses. The contamination rate of the shell may range from 5% to 7% and of the contents up to 0.3% (101).

**Diagnosis**

**Clinical and pathological signs**

Clinical and pathological signs are not pathognomonic. The signs resemble those of pullorum disease (see above). Gross lesions include coagulated yolks, congested liver and spleen and necrotic foci in these organs and caecal cores. In infection with *S. Enteritidis*, pericarditis and polyserositis are also common. Arthritis may also be one of the sequelae. For a full diagnosis the organism must be isolated. Serology on parent birds or the birds themselves, depending on age, may also be indicative.

**Bacteriology and other culture methods**

The established methods recognised internationally for diagnosis have been well documented. Bacteriological confirmation of infection should use standard methods (71, 183). These can involve a pre-enrichment stage in buffered-peptone water to enhance viability (if the organisms are likely to be less viable, for example in dry feed), followed by selective enrichment in liquid media such as selenite or tetrahionate broths or RV medium (183). Standard plating media include brilliant green agar and its various modifications and desoxycholate agar. Bacteria are identified biochemically and serologically.

Improvements in culture methods are continually being made, both to accelerate the process of isolation and identification of *Salmonella* organisms in feed and poultry samples and to make more accurate cultural identification on solid media. Thus, xylose lysine desoxycholate (XLD) medium (156), originally developed for use with *Shigella*, has been useful for identification of *Salmonella* (particularly *S. arizonae*) and *Klebsiella*. However, *Citrobacter* strains may produce colonies resembling *Salmonella*. Not all salmonellae produce hydrogen sulphide and thus would not produce colonies with black centres. Rambach agar (139) has been developed, based on the ability of many *Salmonella* strains to ferment propylene glycol, which produces red colonies with neutral red. A chromogen indicator for β-galactosidase activity can be included to differentiate lactose fermenters. However, *Citrobacter* species may again cause problems. Garrick and Smith recently found that 23/25 *Salmonella* strains produced typical reactions and morphology on Rambach agar while 17/25 did so on XLD (79).

Many developments in enrichment culture have been made for the food industry but these are also relevant for analysing poultry feed components (43). Attempts have been made to reduce incubation times in selective enrichment medium to 6 h-8 h, but reports of success have been conflicting. Some authors report results comparable with those produced by conventional methods while others find a high number of false negative results. A membrane filtration method has been developed with a 6 h enrichment stage (72); a portion of the enrichment broth culture is filtered through a hydrophobic grid membrane which is then incubated on EF-18 agar (73). The hydrophobic membrane reduces the high level of background flora, but colony purification is sometimes necessary. Nevertheless, this method has received official first action status from the Association of Analytical Chemists (AOAC) International (3).

Enrichment serology has been developed in an attempt to combine specificity and sensitivity. Enhancement of flagella production by incubation for 24 h in M-broth, containing D-mannose, prior to examination with polyvalent flagella antisera, produced 95% of the total positive isolations of *Salmonella* obtained by conventional plating (46). In addition, *Salmonella* specific antibodies may be coupled to coloured latex particles. However, at least $10^7$-10$^8$ colony forming units (CFU)/ml are required to induce agglutination (54) and *Citrobacter freundii* can also produce false positive reactions. Several kits are available commercially in Europe and the USA, most of which have the ability to detect 75% to 88% of the samples found positive by conventional culture.

Rappaport-Vassiliadis medium in semi-solid form has been used. Drops of pre-enrichment culture are transferred onto the medium. If migration occurs, slide agglutination is performed (67). The use of anti-flagella serum on a disc incorporated in the agar to specifically identify swarming
salmonellae, followed by plating on Rambach agar was found to give reliable results within 48 h of the commencement of culture (65).

Rapid methods have also been developed to accelerate identification by using a combination of biochemical characteristics and conductance. Conductance methods have been increasingly found to produce results which are comparable with standard culture methods (85). A method involving pre-enrichment in buffered peptone water containing lysine and glucose, with sub-culture to selenite-based media also containing lysine or dulcitol, whose conductance was then measured, has been found to produce results comparable with those produced using standard methods and has been adopted as an AOAC International first action method (3).

A number of immunoassays have been developed for the detection of salmonellae in pre-enrichment or selective-enrichment media (43). A number of different systems have been developed in which polyclonal or monoclonal antibodies have been used, largely in antigen-trap type assays. A solid surface, frequently a microtitre plate, has specific antibodies bound to the well surface. These then trap the organisms in the culture. A reaction is then produced by a further layer of conjugated antibody with a chromogenic substrate. A number of commercial kits are available which perform well under laboratory conditions. Sensitivity is generally limited to a bacterial density of not less than $10^5$ CFU/ml. With field samples, false negative results occur, but the bigger problem is false positives (<50%) produced by antigenically similar organisms (92). Other solid surfaces, such as dip-sticks, magnetic particles or polystyrene beads can also be used. Thus, the problem of this method is the relatively low sensitivity and specificity.

Gene probes may be used for the detection of Salmonella organisms in carcasses or feed. A number of probes developed over the last ten years have been developed from labelled DNA fragments from S. Typhimurium. Good results have been obtained with selective enrichment cultures, but not with pre-enrichment cultures (75). The specificity can be high, but exhaustive surveys of related organisms have not been undertaken. Sensitivity is limited by the amount of DNA or organisms present in the sample. For samples containing small amounts of bacteria, the amount of target DNA must be increased either by choosing rDNA as the target (133), since this is present in high copy number, by culture or by the PCR. The advantage of this latter system is high sensitivity. However, PCR cannot differentiate between dead and living organisms so that an additional stage of pre-enrichment coupled with a comparison of the reaction with standards is necessary to produce a semi-quantitative test. This works well with some biological samples such as chicken skin (110), but not with faeces which usually contain inhibitors for the Taq polymerase. Two or more probes may be used simultaneously to increase specificity.

In many of the methods described above, the time saved may amount to several hours, but for samples such as feeds, preliminary pre-enrichment is still required. The cost of many commercial kits may be prohibitive and initial capital expenditure may be required. In the long term, PCR technology seems to offer the greatest potential for rapid, specific and sensitive assays, but the method is far from being routine laboratory technology.

**Serology**

A number of assays based on agglutination or complement fixation have been used over the years. In some cases, the sensitivity of these has been increased by using antiglobulins (56, 63). As will be seen below, all the systems suffer from relatively poor sensitivity and become cumbersome and less specific when sensitivity is increased.

The crudest of tests based on slide agglutination has been used very successfully with poultry (see above) to eliminate carriers of S. Pullorum. The use of rapid agglutination was introduced by Runnells et al. (141) and a stained antigen whole-blood combination was developed by Schaffer et al. (142). This was introduced in the UK by Gordon and Brander (86) and shown to be of great value in eliminating carrier birds. Retesting at two to four week intervals is recommended until two consecutive negative results are obtained with the whole flock (168). False positive reactions can occasionally cause problems and the whole blood test has been found to be unreliable in turkeys and ducks. This may occur because of infection with other salmonellae, but in many cases the cause is unknown. Other bacteria, including members of the Enterobacteriaceae and enterococci, have also been reported to produce cross-reactions (78). Such problems are likely to be more significant as the incidence of true positive reactions declines.

The test has a number of other disadvantages besides cross-reactions. The results vary depending on antigen quality which may vary with the strain of S. Pullorum and is dependent on operator expertise. Gast found that slight differences existed in results between different stained antigen preparations, although birds infected with the different variants of S. Pullorum were unlikely to be missed (80). Whether large scale screening is cheaper than micro-tests such as the enzyme-linked immunosorbent assay (ELISA) is questionable because the former is labour-intensive; however, one advantage is that seropositive birds can be culled immediately.

The slide-agglutination, whole-blood test for S. Pullorum has been adapted for use with S. Enteritidis, using saponin-containing-antigen to lyse the blood and produce a clearer result (53). Chart et al. found a reasonably good correlation between the results obtained by slide agglutination
and immunoblotting and those obtained with an indirect ELISA, for sera from an S. Enteritidis-infected flock (52). However, exceptions were observed in that some non-agglutinating sera produced relatively high optical density (OD) values, while two agglutinating sera produced low optical densities. Further differences between the results produced by slide agglutination and ELISA were found in a subsequent study where, in five flocks infected with S. Enteritidis, a poor correlation was found for individual sera although high and low OD values in the ELISA and positive and negative agglutinations were found in each of the five flocks (51).

The relative merits of slide agglutination and ELISA for detecting flocks infected with S. Enteritidis have been discussed extensively in published correspondence (22, 53, 62). Slide agglutination may also preferentially identify birds producing immunoglobulin M (IgM). The proportion of birds producing IgM will be relatively low and this isotype does not persist at high titre, but may assist in identifying current infections.

Agglutination-based assays may be more sensitive in the early stages of infection when IgM titres are highest. For poultry, tube agglutination tests have been found to be unreliable with S. Bredeney and S. Virchow (150) and titres against other serovars were found to vary widely (125, 151). In contrast, the indirect haemagglutination test is more sensitive, and specific antibody titres against LPS may persist for several months at titres in excess of 1:100 (150).

Progress in the serological detection of Salmonella infection in poultry has also been made over the last few years. As with earlier technology based on agglutination methods, the newer techniques are applicable mainly to those serotypes and strains that are invasive and induce the production of circulating IgG, including S. Enteritidis, S. Typhimurium, S. arizonae, S. Berta and S. Gallinarum/S. Pullorum. Fortuitously, these are also the cause of major public and animal health problems.

With increasing interest in poultry meat- and egg-derived Salmonella infections in humans, national and international legislation has been introduced (55) requiring frequent monitoring of breeding flocks. Bacteriological methods were the standard procedures, but following increasing international interest in monitoring such infections by ELISA (33, 183), the European Commission has sanctioned the use of ELISA technology for this purpose.

The advantages of serology are that circulating IgG is persistent and avoids the sampling problems arising from intermittent faecal excretion. The major disadvantage is that soon after infection, serum IgG concentrations may be low, whereas faecal excretion will be high. However, use of the ELISA as a flock test should minimise this problem. Non-invasive serotypes will also remain undetected. The advantages of the ELISA over agglutination and microagglutinin techniques relate to ease of procedure and cost.

Two basic systems are available, the indirect ELISA (23) and the competitive 'sandwich-type' ELISA (172).

The indirect ELISA involves the use of a detection antigen coated onto the wells of a microtitre plate. After the application of a blocking reagent to reduce non-specific binding, test samples are applied to the wells. Specifically bound antibody in the sample is detected by an antibody-enzyme conjugate. A variety of antigens, including LPS, flagella, SEF14 fimbriae and crude antigen preparations have been used.

The competitive sandwich ELISA employs a specific mechanism of coating antigen to wells, namely monoclonal antibody. This is then followed by a pure or crude antigen preparation. Test samples are applied followed by conjugated monoclonal antibody which will not bind to the antigen if the test sample contains antibodies with the same specificity. The assay can be shortened by adding both test sample and conjugate together. Monoclonal antibodies have been prepared for flagella, LPS and SEF14 for S. Enteritidis (159, 172).

Both systems have advantages and disadvantages. The indirect assay is simpler and reagents are available for all Salmonella serotypes in chickens, turkeys and ducks. The competitive ELISA can be applied to all animal species and in general shows higher specificity. However, the reagents are not commercially available for all serotypes. Some affinity problems have also been encountered and competitive ELISA may be less sensitive than the indirect assays. In the field, both systems have been known to produce false positive reactions.

A number of detecting antigens have been used. Lipopolysaccharide is the most frequently used antigen. A certain degree of discrimination can be made between sera from chickens infected with different serogroups such as B, C1, C4, D, E1 and E4 (52, 91). However, varying degrees of cross-reaction occur in the case of groups B and D due to the common 12 antigen (52). The problem can be reduced by adjustment of the test sample dilution or by mild periodate treatment of group D LPS which destroys cross-reacting epitopes while preserving the 09 specificity (171).

Flagella antigens have also been used to avoid the problem of cross-reactions. Unfortunately, no information is currently available on the cross-reactions produced by in vivo expression of phase 1 and phase 2 flagellar antigens. Under experimental conditions using S. Enteritidis infections (16) or S. Typhimurium infections (91), specific immune responses can be demonstrated. However, in the field, confusing
cross-reactions seem to occur. Flagella-specific IgG is also reported to be less persistent than LPS-specific IgG; detectable quantities disappear within four months, and the titres peak before those of LPS-specific antibody. Not all chickens produce high titres of flagella specific antibody (165). One area where flagella antigen can already be used with success is to differentiate between infection caused by flagellate and non-flagellate serotypes, particularly *S. Enteritidis* and *S. Gallinarum/S. Pullorum*. Timoney *et al.* were able to differentiate these infections in this way (165). Barrow *et al.* found similar results using ELISA and immunoblotting (32). Birds in a flock in Brazil, in which clinical typhoid was observed, were identified by high IgG titres to group D LPS and by low gm-H specific IgG titres. Thorns *et al.* have used indirect and capture ELISAs using the *S. Enteritidis* fimbral SEF14 antigen (159). Specific IgG was detectable one week post infection and persisted for at least eight weeks when the experiment was terminated. No cross-reactions were observed with sera from birds infected with *S. Typhimurium* or *S. Gallinarum*. A capture ELISA was used to examine field sera and was compared with an indirect ELISA using flagella or heat-extract antigen. The SEF14 antigen ELISA was as sensitive as the heat-extract antigen ELISA and as specific as the flagella ELISA.

Egg yolk may be used in place of sera (64, 109, 124). A standard indirect ELISA may be more appropriate for analysing egg yolk than a competitive (blocking) sandwich ELISA. The low dilution of the samples used in the latter ELISA may lead to interference and to reduced sensitivity and false negative reactions (170).

Serum or blood may be dried onto absorbent paper and eluted at a later date with no loss of titre. Antibiotic therapy for recent infections may result in lower antibody titres. Vaccination of birds could also induce production of cross-reacting antibodies.

Increasing evidence is available to suggest that ELISAs are useful for screening birds in the field for infection with *S. Typhimurium*, *S. Enteritidis* and *S. Gallinarum/S. Pullorum* (109, 124). However, a number of problems remain. These relate both to the choice of a cut-off OD value whereby sera from infected flocks may be differentiated from those from uninfected flocks, and also to the number of samples that should be taken.

Selection of a cut-off point (OD) from the values obtained with sera from uninfected specific-pathogen-free (SPF) birds may result in a proportion of field sera appearing positive. The use of commercial birds, free of infection, to provide negative control sera should avoid this problem. However, another approach is to choose a cut-off point after observation of the distribution of OD values from infected and uninfected flocks. For *S. Enteritidis*, the profiles are very different (24) and for this serotype at least, and probably also for *S. Gallinarum* and *S. Pullorum* (A. Berchieri and P.A. Barrow, unpublished data), a much higher cut-off OD value may be chosen to avoid false positive reactions. The number of samples taken at each time point should ideally be higher than the number (sixty) mentioned in legislation (24). For example, testing 300 samples on each occasion would produce greater confidence in detecting lower prevalences of infection.

Thus, ELISAs have been shown to be very useful for large scale screening of poultry flocks, and this can be performed with very little capital outlay. Screening using ELISAs has been accepted both by the European Union (EU) and by the WHO for use with *S. Typhimurium* and *S. Enteritidis* and could also be used to replace the rapid slide agglutination test for *S. Pullorum* and *S. Gallinarum* wherever possible.

### Public health implications

#### Antibiotic usage

Antibiotic chemicals are used for a number of reasons. These include chemotherapy of *Salmonella* and other bacterial infections (including *E. coli* and *Mycoplasma*), reduction of faecal carriage of *Salmonella* and growth promotion/stimulation (sometimes now referred to as digestive enhancers). The list of antibiotics used is long and varies according to the country involved and the regulations which restrict general use without veterinary prescription. In many Western countries, antibiotics can only be used under the supervision of a veterinarian, whereas in many other countries antibiotics are freely available. Despite the relatively extensive use of penicillin derivatives, and more recently, fluoroquinolones, the incidence of multiple antibiotic resistance in *Salmonella* from poultry has always been very low, in contrast to the situation in the calf rearing industry where over-use has led to the gradual evolutionary development of strains of *S. Typhimurium* resistant to several antibiotics, many of them on self-transmissible plasmids (161), but with additional chromosomally mediated quinolone resistance (163). Such strains have occasionally been isolated from poultry but have not become widespread.

Chemotherapy may be used in very young chickens where serotypes such as *S. Typhimurium* and *S. Enteritidis* can produce mortality. A variety of antibiotics are used for this purpose. In addition, the problem of *S. Enteritidis* in broiler breeders or layers has led to attempts to reduce the frequency of egg contamination by chemotherapy immediately prior to stock movement from rearing to laying accommodation (where this occurs), followed by restoration of gut flora by oral administration of a competitive exclusion preparation. Tetracyclines, furazolidone and fluoroquinolones have been used for this purpose. Furazolidone is no longer used within the EU because of the potential danger as a carcinogen, but is still used elsewhere.
The concern over the use of antibiotic therapy has always been one of the selection of resistant clones or the encouragement of transfer of resistance in the intestine. A number of experiments have been performed in which a genetically marked strain (gyrA conferring resistance to nalidixic acid) of S. Typhimurium was inoculated orally into four-day-old chickens, and the faecal excretion monitored over several weeks (146). A control (non-medicated) group and medicated groups were reared on different antibiotics (500 mg/kg, except for trimethoprim at 100 mg/kg) for a period of nine days. The unmedicated chickens excreted the inoculated strain for five weeks. With the exception of a combination of trimethoprim and sulphadiazine (which produced a profound reduction), none of the antibiotics tested (furazolidone, neomycin, spectinomycin, polymixin, streptomycin, chloramphenicol, tetracycline and ampicillin) produced a major reduction in faecal excretion of the inoculated strain during the period of chemotherapy. The absence of an effect with chloramphenicol was not surprising because very little is present in the lower bowel after oral administration. Following the withdrawal of the medication, the excretion rates in birds given trimethoprim/sulphadiazine increased. This was because the antibiotics were active not only against the Salmonella but against other commensal members of the gut flora which are themselves relatively inhibitory to colonisation by Salmonella. This is the rationale behind the oral administration of a gut flora preparation immediately after chemotherapy is withdrawn. The elimination of these organisms thus increases susceptibility to reinfection from the immediate environment of the birds and increased multiplication of Salmonella occurs until the gut flora is fully restored. When the E. coli flora from these birds were also examined, the initially sensitive E. coli flora were replaced in a few days by a resistant strain of E. coli, particularly in the group administered ampicillin, but also in those administered streptomycin, sulphonamide, tetracycline, chloramphenicol and spectinomycin. After a few weeks, this resistance, present by virtue of a self-transmissible plasmid, transferred to the S. Typhimurium. This highlights the dangers of antibiotic therapy of Salmonella in poultry. Additional experiments with ampicillin and neomycin, the latter in an attempt to mimic attempts to eliminate Salmonella from the gut immediately prior to slaughter, produced similar results (145). The use of gentamicin in some countries (90) is potentially highly dangerous from this point of view.

Additional experiments with S. Enteritidis and enrofloxacin also demonstrated the rapid development of resistance in the E. coli population. Although this resistance is thought to be always a chromosomal mutation, and therefore not transmissible normally by plasmids, resistance may be transferred between certain Salmonella strains by transducing bacteriophages (35).

Until 1969, low levels of chemotherapeutic antibiotics, in addition to copper sulphate, were used as growth promoting agents (4). Prior to this, a report from the Netherlands (the Netherhope Committee of the Agricultural and Medical Research Councils) reported that the practice was safe. However, in the UK, the appearance of multi-resistant S. Typhimurium in calves was perceived to be a very worrying development and the use of such drugs for this purpose was investigated with the result that the use of chemotherapeutic agents as growth promoters was banned in the UK (with the exception of zinc bacitracin which, at the time, was used purely topically in human medicine). This led to similar bans in the EU, but not so far in the USA. Pharmaceutical companies subsequently developed new antibiotics which promoted growth without any direct effect on Salmonella or E. coli. The spectrum of activity of the antibiotics was different. However, because they affected members of the gut flora which were themselves inhibitory to Salmonella colonisation, these drugs altered the susceptibility of poultry to infection with Salmonella. Some, such as tylosin, nitrovin and the glycopeptide, avoparcin, considerably increased faecal excretion of Salmonella (26, 147). The effect can be reduced by using lower concentrations in the feed (19). In addition to the effect on Salmonella, these antibiotics also select for resistance to glycopeptides, such as vancomycin, in Enterococcus faecium (174). This is potentially a very great public health threat, since the transposon can transfer the resistance to multi-resistant strains of Staphylococcus aureus (127) and since poultry and pig strains of E. faecium can colonise the human gut (38), the development of vancomycin resistant S. aureus concerns those involved in public health. Some argue that no evidence exists of a risk to public health. Presumably those presenting this argument would only be convinced when transfer of vancomycin resistance to already multi-resistant S. aureus had occurred. This may be seen to be an irresponsible attitude by many others involved in public health and consumer issues. The use of growth promoting antibiotics, including avoparcin, has now been banned in the EU. However, this action, without controlling import of poultry and poultry meat from outside the EU, does not completely solve the problem. The pharmaceutical industry will continue to sell these compounds to countries where restrictions do not exist. Poultry produced in these countries may still be sold to countries with restrictions. Regulation world-wide, although currently impossible, appears to be the most logical way to regulate this problem.

**Legislation and public profile**

The high public profile of Salmonella food poisoning in many countries during the 1990s has arisen largely from the epidemics of S. Enteritidis in poultry. In the UK, particularly, the response to inappropriate statements by ill-informed politicians created a feeling of unease and helplessness in the public. Public Health Laboratory Service reports which indicated that eggs and egg products were the main vehicle of the infection resulted in a panic response on the part of the consumer in the form of reduced purchase of eggs. This had a direct effect on the laying side of the industry in the UK, resulting in incomes being reduced and many farms going out...
of business. An indirect effect was that imported eggs were seen by the public to be healthier than those produced in the UK, even though, at the time, no way of testing for the presence of *Salmonella* existed, either in the eggs or, easily and quickly, in the birds themselves.

The political response in the UK was also panic-driven and inappropriate legislation was introduced (6, 7, 8, 9) to increase monitoring of breeder farms and laying farms and to increase the powers of the Ministry of Agriculture, Fisheries and Food (MAFF), allowing slaughter and any other control measures for infected farms that were deemed to be suitable. Attempts were also made to improve monitoring and control of protein supplements imported into the UK for use in poultry feeds. Several years later, when data began to be collected and published on the incidence of *S. Enteritidis* in broilers and broiler breeders, broiler meat and not eggs was found to be responsible for exposure of the public to *S. Enteritidis* infection in the majority of cases. Some of the legislation was repealed as it was shown to be ineffective and unsuitable.

These events show how panic reactions to public health issues do not necessarily result in the most suitable control measures being employed, particularly when information on which decisions can be made is unavailable. This scenario was repeated several years later in the UK with bovine spongiform encephalopathy.

However, the publicity in the UK and the Netherlands where a similar approach was taken (68) prompted the EU to introduce the Zoonoses Directive (55), which has led to an attempt at a top-down approach to controlling *S. Enteritidis*, primarily in breeders and then, should this be successful, in layers and broilers. Whether this will be successful or not depends, to some extent, on the political will to address the problem. Greater success has been obtained in parts of Scandinavia, particularly Sweden, where following a large outbreak in the 1950s, stringent control measures were introduced for farms where *Salmonella* infections were found. These comprised a test and slaughter scheme with use of a competitive exclusion product. Compensation was initially paid to farmers where control measures were introduced, which included slaughter of flocks. This has, over the years, been substituted by the use of insurance schemes. The greater success in Sweden reflects the generally smaller size of the poultry industry in that country compared with those in other countries of western Europe (175).

Other countries within the EU have adopted different schemes for tackling the *S. Enteritidis* problem. In East Germany, prior to reunification, live vaccines for a number of *Salmonella* serotypes were developed. These were generally auxotrophic, produced by chemical mutagenesis and were sometimes antibiotic resistant. When used with management changes, the vaccines were shown to be effective against *S. Dublin* in cattle, and vaccines for *S. Typhimurium* and *S. Enteritidis* were also produced. After reunification, these vaccines were adopted by the new Federal Government for use throughout the country. Additional live vaccines, produced in a similar way, are also available in Germany. Both are required to be used in layers during the rearing period in that country. Breeders are also vaccinated with these live vaccines and with a killed vaccine, although this is not required by law.

Legislation in the USA has occurred step-by-step. Despite the adoption of the National Poultry Improvement Plan, an improvement in the levels of *Salmonella* contamination in poultry was not a target until recently. However, recent outbreaks of infection with *Salmonella* and *E. coli* O157 have concentrated the minds of the legislators, resulting in the requirement of processing plants to attain *Salmonella* incidence standards on processed boilers and to produce pathogen-free meat (12). This exerts pressure on poultry producers which might not be possible in any other way.

Different countries have approached control in different ways, and comparison of the experiences of each will assist in determination of the most efficient means for control.

**Trade implications**

*Salmonella* serotypes responsible for paratyphoid in poultry and gastroenteritis in man are ubiquitous in the environment and are distributed worldwide in poultry. Some exceptions occur, for example, *S. Enteritidis* has not become widespread in poultry farms in Australia. This indicates that restricting the introduction of new serotypes should be possible, at least in those cases where natural geographic barriers exist. A combination of bacteriological monitoring and serology will provide information on the infection status of stock being introduced to a country. The latter is necessary because of the risk of antibiotic therapy prior to shipment. A combination of these measures and strict hygiene and staff management will delay or even prevent introduction of new strains. This is, however, dependent on the housing and ambient temperature.

Assessment of the relative risk of different chicken products is always difficult. The risk will relate to the standards in the poultry industry in each country. Extrapolation may be made from those countries where some information is available. Breeding stock are generally reared in high quality environments and these and hatching eggs represent a lower risk of infection than chicks where considerable horizontal spread of infection may occur and where fluctuations in ambient temperature may cause stress. Contrary to popular belief, table eggs represent a much smaller risk of infection than chicken meat, whether this is whole carcasses, sections or deboned meat. This is borne out by the incidence of infection in laying flocks, broiler flocks and table eggs (138).
Biological prevention and control methods

Antibiotic usage

As indicated above, antibiotics are used for chemotherapeutic and growth promoting purposes. This is effective, but additional side effects occur, namely the selection for antibiotic resistant bacterial strains and promoting the in vivo transfer of antibiotic resistance and in some cases, increasing intestinal colonisation and faecal excretion of *Salmonella*. These negative aspects must therefore be considered when such chemicals are used to treat poultry which might be infected with *Salmonella*.

The situation regarding the use of growth promoters in the EU has been unfortunate, since the antibiotics recommended by the Swann report (4) were not used for therapy in human or veterinary medicine at that time, and were only introduced for this use some years after being used as growth promoters. However, the separation of antibiotics that may be used for therapeutic and growth promoting purposes makes good sense from the public health point of view, whilst the negative economic effects on the poultry industry are unfortunate. These negative effects must be considered since, as indicated above, this could result in the importation of animals or meat which can be produced more cheaply in other countries where such restrictions are not in force. The withdrawal of some growth promoters is also thought to have side-effects on the incidence of clostridial necrotic enteritis. However, this may be controlled therapeutically with antibiotics rather than prophylactically, as was the case with growth promoters. Some producers are addressing these problems and testing the use of alternative feed additives in association with changes in management practices.

Additional concerns have arisen recently over the inexorable increase in resistance to chemotherapeutic antibiotics in enteric and other bacteria. This concern is currently restricted to Europe. In comparison, there has been little concern in the USA (169). In the UK, the Swann report was mainly concerned with the use of antibiotics as growth promoters and use in veterinary medicine, either therapeutically or, more importantly, prophylactically, was not considered. This has always been a very difficult issue, the regulation of which has not been addressed by any of the major reports on antibiotic resistance (13, 14, 119, 184). Other than recommending increased surveillance, few concrete suggestions were made, and few of these are likely to have any effect. The Advisory Committee on the Microbiological Safety of Food recommended a number of measures to increase controlled use of antibiotics, none of which would markedly affect usage since this would be left in the hands of the veterinary profession (14). A MAFF report has recommended use of fewer antibiotics and improved management of use, together with the increased use of disinfectants and vaccines (119). The House of Lords Committee advocated setting up a multi-departmental government committee to oversee all aspects of antibiotic use (13), a recommendation of the original Swann report. The WHO report reflects the multi-national composition of those involved in defining recommendations (184), most of which reflect those already mentioned above. Such recommendations have also been put forward by the pharmaceutical industry itself (15), particularly that use should be restricted to recommended uses. However, a number of issues are not tackled. These include the following:

a) that sentinel bacteria such as *E. coli* and a Gram positive micro-organism might be used for monitoring resistance. Such omnipresent bacteria would be more useful than studying resistance in pathogens, which might not always be present

b) the evolutionary pressures exerted by antibiotics used prophylactically. Thought should be given to a restriction of this use with only therapeutic use being allowed

c) that antibiotics should perhaps not be used to reduce intestinal carriage of food-borne pathogens

d) that trade controls might be introduced to prevent importation of poultry and poultry meat which is considered to pose potential public health risks due to the use of growth promoting antibiotics which are banned in the country/region in question

e) that greater emphasis should be given to the use of disinfection and vaccination of dams to reduce the problems of morbidity and mortality in young poultry which require medication.

The use of antibiotics remains an important issue for public health in addition to animal health and economics. The issues raised must continue to be addressed by those countries concerned and should also be considered by countries which do not currently regard this issue as being of importance.

Competitive exclusion

Experimental inoculation of newly hatched chickens with *Salmonella* organisms results in heavy excretion of large numbers of the inoculated bacteria for a long period of time. Inoculation of adult birds results in excretion of smaller numbers of bacteria for shorter periods. The reason for this difference is that the adult has a high degree of protection conferred by the presence in the alimentary tract of a complex intestinal microflora. Disruption of this flora can result in increased excretion of *Salmonella* (146). As a corollary, if the gut flora of the adult is administered to newly hatched chicks, the young bird has the same resistance to colonisation possessed by the adult within 24 h (128). The poor resistance of the newly hatched chick and poult is largely the result of the high level of hygiene in the hatchery, with the result that several weeks are required for the young bird to acquire this flora naturally (113).

This concept has been applied to attempt to increase the resistance of young poultry to *Salmonella* colonisation. Suspensions of faeces, caecal contents or broth cultures of
When tested in the field, the results have been more involved. Of the untreated control flocks, 24.1% became infected, eight million broilers in 284 flocks on forty-six farms were infected which may spread prior to treatment. Pivnick and Nurmi (136) obtained equivocal results whereas Goren et al. (87) obtained good results. In the trial performed by Goren et al., eight million broilers in 284 flocks on forty-six farms were involved. Of the untreated control flocks, 24.1% became infected with Salmonella, compared to 14.7% of the treated flocks. The proportions of carrier birds were 3.5% and 0.9% respectively. Evidence of hatchery-derived infection was found in this study. Blankenship et al. (44), in a trial in which extensive hatchery infection was also evident (50% of egg shell samples from the hatchery were infected), found 11% infection in control birds and 2% in treated birds, which translated into carcass contamination rates of 41% and 10% respectively. Evidence of effectiveness has also been reported in Sweden (176) and the Netherlands (45, 66). In the former study, where no hatchery problem existed, none of the treated flocks were infected, while 24% of the control birds were infected (in a study of more than one million broilers on forty farms). In the latter study, there was also evidence of an effect against an existing infection. Problems of transfer of pathogens to poultry by the use of competitive exclusion flora have been largely exaggerated and are not significant, provided that care is taken to ensure their freedom by repeated passage of the preparation and screening for bacterial pathogens. Preparations from conventional birds are generally more protective than those obtained from SPF birds. However, such preparations can be inoculated into SPF birds and the existence of pathogens monitored by antibody testing.

Experimental studies have also showed effectiveness against C. jejuni, Clostridium perfringens, C. botulinum, Yersinia enterocolitica and E. coli O157. The extent to which this is relevant to the degree of protection that might be obtained in the field remains to be seen, since C. jejuni and the clostridial infections generally do not become established until the bird is a few weeks old, by which time the bird may have acquired a flora naturally, suggesting that in the field these pathogens may be able to colonise birds with a mature gut flora. However, the effectiveness of competitive exclusion preparations against C. jejuni may be reduced by storage and exposure to the air, suggesting that one of the microbial components is oxygen sensitive. One trial against C. jejuni with 200,000 broilers in fifty-eight flocks demonstrated a degree of effectiveness, with 62% of control flocks and 41% of treated flocks becoming infected. The figures for infected caecal contents were 40% and 21%, respectively (121). Competitive exclusion remains a well tested means by which young birds can acquire a considerable degree of resistance to Salmonella and other food borne pathogens.

**Vaccination**

On account of the lack of information regarding colonisation and immunity relating to the Salmonella serotypes that are usually associated with human food-poisoning, the development of vaccines against non-host specific serotypes for use in poultry has been almost exclusively empirical. In assessing the following studies, consideration of the infection model used is important. The outcome of experimental infection can vary greatly depending on the strain of Salmonella used for challenge, the route and bacterial dose used for inoculation and the age of the bird at the time of inoculation.
vaccination and challenge. Infection with a wild-type strain within a few hours of hatching may realistically reflect infection arising from a hatchery, whereas infection of birds that are several weeks old may reflect infection by a number of routes. Infection of the younger birds by the oral route results in heavier faecal excretion for a longer duration than occurs in older birds. Challenge with a large dose, such as log$_{10}$ 9 CFU may produce a consistent infection but may not reflect the normal situation in the field and may not accurately portray the efficacy of a vaccine against the size of the challenge dose likely to be experienced in the field. A more accurate assessment could be made by challenging a small proportion of the vaccinated and control groups and monitoring the rate of lateral spread of infection, or by the incorporation of small numbers of Salmonella organisms in the feed.

Various types of non-living vaccine have been used experimentally and in the field, and although these generate an immune response, protection is variable. Earlier work indicates that protection is generally no better than moderate. McCapes et al. (108) demonstrated reduced mortality (from 85.6% to 39.7% and from 91.5% to 44.6%) in day-old turkeys challenged via the yolk sac with S. Typhimurium and S. Schwarzengrund, respectively; the parents of the chicks had been exposed to S. Typhimurium infection early in life and vaccinated repeatedly sub-cutaneously with S. Typhimurium bacterin. A less pronounced reduction in mortality (from 94.4% in controls to 75.1% in vaccinated birds) was obtained in experiments using crude endotoxin extract from S. Typhimurium administered intraperitoneally. When young chickens were vaccinated directly, using boiled sonicates of several serotypes incorporated in the feed, then challenged a few weeks later, clearance from the faeces of the challenge serotypes was generally more rapid than for unvaccinated controls (166), although the results were variable. In contrast, Bisping et al. found that orally administered heated whole cell bacterins had little effect on faecal excretion of Salmonella in the faeces (42). Similarly, vaccination of hens with a bacterin did not significantly reduce excretion of S. Hadar from the challenged progeny (158). Parenteral vaccination with formal-killed bacteria, but also challenging parenterally either with S. Virchow (84) or S. Enteritidis (164), reduced mortality and intestinal colonisation. Ghosh also reported reduced mortality from 85% to 0% against log$_{10}$ 7.7 S. Virchow organisms in young chicks, when inoculated intraperitoneally (84). Timms et al. showed reduced mortality from 100% to 50% against between log$_{10}$ 5 and log$_{10}$ 8 challenge organisms in similar experiments (164). Since the increase in human food-poisoning associated with S. Enteritidis in the late 1980s, a number of studies have been undertaken which have vaccinated hens with this serotype killed by different methods. Gast et al. demonstrated reductions in the rate of faecal excretion when birds were challenged two weeks after the second of two sub-cutaneous vaccinations, but not if challenged six weeks after the second vaccination (81, 82). Fewer numbers of the challenge strain were isolated from the spleen, ovaries and oviducts compared to controls. Reduced excretion of Salmonella in faeces was also observed when hens were vaccinated with acetone-killed S. Enteritidis bacterins mixed with Freund's incomplete adjuvant (17). This vaccination induced high levels of circulating specific IgG to unspecified salmonella protein antigens. Nakamura et al. also showed reduced excretion in faeces and reduced numbers of bacteria present in the tissues of birds vaccinated twice and challenged at laying age (123). Currently, a killed S. Enteritidis vaccine is available commercially and is used extensively in national schemes in layers, both in the UK and Germany and possibly other countries. Data are being generated on the use of this vaccine, but have not been published.

Some live vaccines are available and others will become available in the next few years. The effectiveness of these vaccines when applied in the field has not yet been established. Live attenuated vaccine strains developed for immunisation against the host-specific serotypes have been of less value in reducing excretion of those serotypes that are non-host specific. Although oral vaccination of day-old chicks with an S. Dublin vaccine strain had little effect in reducing the faecal excretion, vaccination did limit the systemic multiplication of an S. Typhimurium intraperitoneal challenge ten days later (104, 105). Rough mutants which have defective LPS biosynthesis have been tested for use in poultry. Salmonella Typhimurium strain F98, which is virulent for chickens, was rendered avirulent by selecting for roughness as indicated by bacteriophage resistance. When challenged, chickens that had been vaccinated previously, either orally or intramuscularly with the mutant, showed reduced faecal excretion of the parent strain (29). A mutant of S. Typhimurium which is defective in the uridine diphosphate (UDP)-galactose epimerase gene, galE, required for LPS synthesis (83), has been shown to be an effective vaccine in mice and cattle (185). Such mutants are rough unless exogenous galactose is supplied. When one-day-old chicks were vaccinated orally with the galE mutant and challenged, albeit only two weeks later, with an S. Typhimurium isolated from turkeys, the number of Salmonella organisms excreted in the faeces, and the number of chicks reaching carrier status, was significantly reduced (137). Protection was more pronounced when chickens were inoculated intramuscularly rather than orally (155). However, galE mutants of S. Choleraesuis and S. Typhi have been found to retain virulence for mice and humans respectively (99, 126), and thus the galE mutation is now generally thought to be less valuable than previously.

An aroA mutant of S. Typhimurium strain F98 was attenuated for chickens and gave protection to four-day-old chicks against challenge with 10$^8$ CFU of the parent strain, when vaccinated by the intramuscular or oral routes (29). Cooper et al. obtained similar results (57, 59). In both cases, the vaccine was administered orally within one day of hatching. The degree of protection was greatly improved when challenge was made by contact infection soon after oral vaccination of
the newly hatched chicks (60). These authors found no evidence of cross-protection between S. Enteritidis and S. Typhimurium in terms of excretion in the faeces. Others have found that a rough mutant of an S. Enteritidis phage type 4 aroA strain protected laying hens against challenge with the wild-type strain, but better protection against S. Enteritidis was obtained by parenteral vaccination with the S. Gallinarum 9R vaccine strain (178). However, the 9R vaccine strain was found to persist in the tissues (31). As mentioned above, a purE mutant strain is being used extensively as a chicken vaccine in Germany (118). A cya crp mutant of S. Typhimurium was shown to be avirulent and immunogenic in chickens. When challenged with the parent strain two weeks after oral vaccination with this mutant, protection was obtained, not only against the homologous serotype S. Typhimurium, but also against S. Enteritidis, S. Agona, S. Heidelberg, S. Breedeny, and some other serotypes including S. Hadar, S. Montivideo and S. Anatum (93). However, the interval between immunisation and challenge was very short and may have led to stimulation of non-specific immunity. In addition, when adult chickens were vaccinated with the S. Typhimurium cya crp strain, maternal antibodies were demonstrated in eggs and progeny, which had reduced colonisation by the wild type strain (94). The workers who performed these studies have also suggested that vaccines that are too virulent may be counter-productive because immunosuppression occurs (95). These results are confusing and unpublished results by these authors indicate that cross-protection and long lasting protection may have been incorrectly reported. In addition, use of the cya crp mutant of S. Typhimurium would cause some concern as it retains considerable virulence (P.A. Barrow, unpublished results).

One additional aspect of the use of live attenuated vaccines against Salmonella relates to the ability to colonise the alimentary tract of very young birds. If such vaccines are administered to newly hatched chicks, the organisms multiply extensively because of the absence of the normal complex microbial flora found in adult birds. This in turn prevents colonisation by other Salmonella strains inoculated within the following few hours (28, 39). This is a purely microbiological effect, and may explain the degree of protection found by Alderton et al. where administration of an aroA S. Typhimurium vaccine produced significant protection when the birds were challenged ten days later (2). Some evidence suggests that this phenomenon does occur in very young birds given live, attenuated vaccines (P. Coloe, personal communication). The inhibition is neither the result of bacteriophage or bacteriocin activity nor a consequence of stimulation of non-specific immunity. Protection may thus be obtained against homologous and heterologous serotypes and phage types. The practical consequences are that live vaccines could be administered to very young chickens by the oral route, thereby obtaining protection within a few hours. Rapid protection would then be obtained by this colonisation-inhibition effect followed by the development of normal immunity within a couple of weeks. However, the choice of strains from which to develop future live vaccines might depend on testing of inhibitory activity, since a number of those currently available have a very poor inhibitory spectrum (116).

The ideal route of administration to poultry would be orally in drinking water or food or by spray. However, parenteral administration may be an additional requirement for maximum protection. Although the ideal vaccine should be avirulent for chickens, oral vaccination may require the use of an invasive strain to stimulate maximum immunity, because immunogenicity may be correlated with invasiveness. Regardless of the route of inoculation, some residual virulence may result in vertical transmission, as occurs occasionally with the S. Gallinarum 9R vaccine. Therefore, the vaccine should produce no disease in the progeny, nor affect productivity, while protection should obviously last as long as possible. Protection of broilers is required for a few weeks, although Salmonella control in broilers is not legally required in the EU. However, control in breeders is an integral part of the control programme in Europe and hence in breeders, and in layers, protection is required for many months. Vaccination should therefore be compatible with the use of competitive exclusion (early application or replacement of gut flora). Following vaccination, a protective immunity requires several days to develop. This delay could be overcome by using a live vaccine strain that in newly hatched birds shows the colonisation-blocking effect, a form of competitive exclusion that occurs between closely related enteric bacteria. Vaccination with competitive exclusion products can produce a degree of protection against salmonellosis in the early life of the young chicks. In this case, immunity is likely to have little protective effect against infections that are extant at the time of immunisation. This might mean that an appropriate live attenuated strain could protect against potential 'ex-hatchery' infection during the first few days of life followed by the development of true immunity. When the vaccine strain has been completely eliminated, limited cross-protection against other serotypes seems likely, although evidence to the contrary has been reported recently. Given the small number of major serotypes involved, this should not be a significant problem. However, this emphasises the lack of knowledge of the principal Salmonella immunogens involved in immune protection. The role of LPS, the feasibility of multivalent vaccines and the contribution of other antigens remain to be elucidated. Only by investigating these areas can truly non-empirical vaccines be generated.

Probiotics

Probiotics are bacteria which may have originated in the gut, or be derived from elsewhere, or other non-viable or viable products which, when administered orally to animals, alter the existing gut flora and thereby produce a beneficial effect in the animal. This benefit is thought to be conferred by alteration of the composition of the intestinal flora such that existing micro-organisms which are inherently harmful are displaced by those which are beneficial, either in terms of
nutrition or resistance to pathogens. A great deal of interest has surrounded this idea for many years (77), and this interest has become intensified in those countries where the use of growth-promoting antibiotics has been limited or prohibited. A number of different products have been tested as growth promoters, such as oligosaccharides, metabolic enhancers, enzymes, oils, nucleotides and organic acids, but the effects of these products on *Salmonella* are totally unknown.

The use of probiotic preparations and organisms on *Salmonella* colonisation has been subjected to few controlled studies. Most of the work has been on broiler and layer performance. However, one report has indicated a positive effect of avian *Lactobacillus* strains in reducing the mortality produced by a high dose of *S. Typhimurium*, which was reduced from 33% in the untreated birds to 14% in those receiving the lactobacilli (173). Other reports have indicated little or no effect on intestinal colonisation and faecal shedding (1, 18, 153).

Hygiene and management

The importance of high levels of hygiene and management in poultry rearing to avoid contamination with *Salmonella* and other pathogenic bacteria cannot be emphasised too highly. The possibility of rearing birds totally free of zoonotic pathogens has been demonstrated by large breeding companies and research institutes. However, at this particular level, the cost of avoiding infection is prohibitive. Nevertheless, a considerable degree of success can be obtained by following a number of rules. In many countries, codes of practice are published by governmental departments which give advice on these areas, and a report has been produced by the WHO following the world-wide egg problem in the 1980s (181). In the UK, several codes of practice exist which pertain to different areas of production, including broilers, breeders and hatcheries and layers. Quality of housing may also be a limiting factor, both in terms of age and ambient temperature, since open-sided housing implies obvious additional potential problems with environmental contamination, and older housing is more difficult to clean.

Within these codes of practice, management schemes for staff and entry of personnel are also important, including restricting entry, appropriate clothing, and washing and cleaning of both staff and clothing. Monitoring of *Salmonella* carriage by staff may also be important. Guidelines for housing and management are also available and are a recognised principal factor in reducing spread of infection (180). Disinfection and sanitation of housing is important. A number of recommended commercial products are available which have been tested with the standard assays for activity in the presence of organic material. However, other studies have indicated that in the presence of organic material likely to be found in houses after depopulation, not all of the products were effective (40). Phenolics were the most effective of the commercial products in the presence of organic material such as feed and faeces. Old wooden material, found as structural components of older housing, also inactivated products. The most effective chemicals were formaldehyde and gluteraldehyde, although the toxicity of these chemicals can present problems.

Les salmonelles paratyphoïdiqes

P.A. Barrow

Résumé

Les bactéries paratyphoïdiqes du genre *Salmonella*, qui comptent plus de 2 000 sérovars et sérotypes, posent un grave problème à l'industrie avicole. Cela est, pour une large part, la conséquence de l'introduction des bactéries dans la chaîne alimentaire. Chez l'homme, l'infection se manifeste sous forme de gastro-entérite pouvant entraîner la mort de sujets très sensibles. Chez les volailles très jeunes, les souches de certains sérotypes peuvent entraîner la maladie et la mort. Chez les adultes, quelques sérovars se localisent dans l'appareil génital avec transmission verticale concomitante. L'association entre *S. Enteritidis* et les œufs de consommation a fait l'objet d'une large couverture dans les médias, suscitant beaucoup d'inquiétude. Cette dernière a été à l'origine des mesures prises aux plans national et international pour lutter contre les principaux sérotypes (*S. Typhimurium* et *S. Enteritidis*), dans les élevages de sujets reproducteurs et de poules pondeuses. La prophylaxie passe par une
hygiène rigoureuse et de bonnes pratiques d’élevage, mais ces mesures ne sont pas toujours supportables sur le plan économique. La même difficulté se pose pour les tests sérologiques et bactériologiques, ainsi que pour l’abattage sanitaire. Les antibiotiques ont été utilisés pour réduire le portage, mais ils peuvent poser des problèmes de résistance et renforcer la sensibilité. Les antibiotiques activateurs de croissance peuvent également accroître la sensibilité à l’infection. Aussi recherche-t-on désormais d’autres moyens de renforcer la résistance des volailles à l’infection. On se tourne notamment vers l’administration aux poussins qui viennent d’éclore de préparations à base de flore intestinale, qui permettent de lutter contre Salmonella par exclusion compétitive. Des vaccins à souches vivantes et inactivées ont été essayés et ont donné quelques bons résultats. Cependant, des doutes subsistent sur l’innocuité de certains vaccins à souches vivantes.

Mots-clés

Salmonelas paratifoideas

P.A. Barrow

Resumen
Las bacterias paratifoideas del género Salmonella, grupo que comprende más de 2.000 serovares o serotipos, crean muchos problemas al sector avícola, sobre todo por la entrada de esas bacterias en la cadena alimentaria humana. En el hombre la infección provoca episodios de gastroenteritis, que en personas muy sensibles pueden llevar a la muerte. Algunas cepas de ciertos serotipos tienen la capacidad de inducir morbilidad y mortalidad en pollos muy jóvenes. En aves adultas, algunos serovares se instalan en el tracto reproductivo y se transmiten luego verticalmente. La frecuente asociación entre S. Enteritidis y los huevos de consumo humano ha tenido gran eco y causado profunda inquietud, dando lugar a diversas tentativas nacionales e internacionales de controlar la presencia de los principales serotipos –S. Typhimurium y S. Enteritidis– en bandadas de aves reproductoras y ponedoras. Aunque es posible controlar la infección aplicando estrictas medidas de higiene y gestión, tales medidas no siempre resultan económicamente viables. Esa circunstancia puede imposibilitar el control de la enfermedad por aplicación de pruebas serológicas y bacteriológicas y el subsiguiente sacrificio sanitario. Aunque utilizados a veces para reducir la carga infecciosa de ejemplares portadores, los antibióticos pueden dar lugar a problemas de resistencia y a un aumento de la susceptibilidad. Los antibióticos promotores del crecimiento también pueden incrementar la susceptibilidad a la infección. Por tal motivo se están buscando otros métodos que confieran a las aves de corral mayor resistencia a la infección, entre ellos el uso de preparaciones de flora intestinal en pollos recién eclosionados para inducir la exclusión competitiva de Salmonella. Se utilizan asimismo vacunas vivas y muertas, ambas con cierto éxito, aunque el nivel de seguridad de algunas de las primeras sigue suscitando dudas.

Palabras clave
References


