Control of food-poisoning salmonella in poultry – biological options

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The high cost of rearing pathogen-free poultry, coupled with increases in importation from non-European countries where hygienic measures may be less stringent indicates that biological approaches to control of infection with food-borne pathogens will continue to be important. The major measures are dealt with in this review, together with the positive and negative aspects of each of these approaches. They include antibiotic therapy and prophylaxis, competitive exclusion using intestinal flora preparations, live and killed vaccines and the increasing interest in exploiting natural genetic resistance to infection and disease.

Keywords: Salmonella; chicken; zoonoses; vaccination; antibiotics; competitive exclusion; genetic resistance

Introduction

Infections in poultry are a major source of human infection although pigs are also increasingly seen as an important source of infection and this is currently being assessed in several countries. The incidence of human salmonellosis has increased greatly over the last 20 years and this can be attributed largely to the epidemics of *S. enteritidis* phage types in poultry in a large number of countries. The reason for the rapid spread of infection with a relatively small number of phage types throughout the poultry industry in several countries is totally unknown. A hypothesis has been suggested that *S. pullorum* and *S. gallinarum* have been replaced by *S. enteritidis* (Rabsch *et al.*, 2000). However, this seems unlikely to be responsible given the recent low incidence of *S. Pullorum* and *S. gallinarum*. The appearance and gradual disappearance of individual strains is a well-known phenomenon and has occurred in previous decades with *S. agona* and *S. Hadar*, amongst others. The current problem with *S. enteritidis* has been largely attributed to the consumption of *S. enteritidis*-infected eggs. However, it is likely that infected broilers are equally or more to blame.

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The entry of infected stock into the food chain is exacerbated by the gross spread and cross-infection that occurs during slaughter (Mead, 1989). The World Health Organisation (WHO) recognises that the three areas where infection control may be sensibly exerted are by education of the public, by improvements in slaughter hygiene and technology and by control of infection in the birds themselves (World Health Organisation, 1980; 1990). There are, however, limitations in the extent to which public education can be effective in this. The economics of abattoir processing indicate that there may also be financial limitations in the extent to which improvements may be made at this stage. It seems likely therefore that, as with past control of other bacterial zoonoses, such as bovine tuberculosis and brucellosis, control in the animals themselves must be central to infection control policy. However, the economics of poultry production must be an important factor in introducing control measures. Profit margins are small and the apparent absolute requirement for free trade will result in those countries which introduce such measures to control infection, such as salmonellosis, being placed at a financial disadvantage in contrast to countries where no measures are taken. Introduction into international free-trade legislation of a public health component and conditions would seem an important measure. This would mean that those countries wishing to improve the bacterial zoonosis status of their national flocks would not be penalised as a consequence.

The epidemiology of *Salmonella* infection in poultry is complex but in summary, the major sources of infection for poultry are the birds themselves, feed and the environment (including housing) (Symposium, 1991). It is possible to rear poultry totally in the absence of *Salmonella*. Large breeding companies and research establishments do it at a high cost. This is done through the introduction of improved housing and diet, together with employment of skilled staff and efficient management structures. In addition, introduction of thorough hygiene and disinfection measures and other schemes to reduce the chances of cross-infection, such as "all in-all out" rearing are required. In some cases these things will be possible and, as existing housing degenerates, requiring replacement, improvements can be made slowly. In countries with high ambient temperatures, open-sided housing may limit the extent to which improvements may contribute to reduce environmental sources of infection. However, the financial incentives to eliminate a food-poisoning pathogen from stock, which has very little direct impact on productivity and for which financial incentives are not available, poses considerable imponderable problems for poultry companies. It seems likely, therefore, for the foreseeable future, that biological control measures will be an increasingly attractive option. This short review aims to present some of the options for biological control.

Antibiotics and chemotherapeutic agents

Antibiotics and other chemotherapeutic agents are used for the treatment and prevention of a number of bacterial diseases of poultry, systemic diseases caused by genera such as *Salmonella* spp., *E. coli* and *Mycoplasma* spp., reduction of faecal carriage of *Salmonella* and growth promotion/stimulation (sometimes now referred to as digestion enhancement). The list of antibiotics used is long and varies according to country and the extent to which national regulations restrict general use without veterinary prescription. Despite the use of penicillin derivatives and, more recently, fluoroquinolones, the incidence of multiple antibiotic resistance in *Salmonella* has traditionally been very low, in contrast to the situation in the calf rearing industry. Strains such as *S. typhimurium* DT104 were originally isolated from calves and have been isolated with increasing frequency in the poultry industry.

THERAPEUTIC AND PROPHYLACTIC USE

Chemotherapy may be used in very young chickens where serotypes such as *S. typhimurium* and *S. enteritidis* can produce mortality. 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, followed by restoration of the gut flora by oral administration of a competitive exclusion preparation. Tetracyclines, furazolidone and fluoroquinolones have been used successfully for this. The major concern over the use of antibiotic therapy has always been one of the selection of resistant clones. Experimental work (Smith and Tucker, 1975) suggests that some antibiotics, including tetracycline, ampicillin and chloramphenicol, have little or no effect on faecal shedding of the *Salmonella*. After medication was discontinued faecal shedding may increase for a while, presumably as a result of the effect of the antibiotics on the normal intestinal flora, which inhibit colonisation by pathogens. Plasmid-mediated antibiotic resistance in the *E. coli* flora may also transfer to the *Salmonella* population under such conditions, confirming that the use of antibiotic therapy leads to the encouragement of transfer of resistance in the intestine. The massive increases in the use of quinolones and fluoroquinolones since the mid 1980's has led to the isolation of an increasing number of *Salmonella* strains which are resistant to nalidixic acid (Piddock *et al.*, 1990; Wray *et al.*, 1990). Despite the fact that, as far as we know, this resistance is always a chromosomal mutation, and therefore not transmissible normally by plasmids, it may be transferred between certain *Salmonella* strains by transducing bacteriophages (Barrow *et al.*, 1998). Its use in poultry has now been banned in the United States of America and it has precipitated considerable debate on its use. Even in the absence of clinical salmonellosis, *Salmonella* organisms colonising the gut, are subject to selective pressure from these chemicals when they are used against other bacterial diseases.

GROWTH PROMOTING USE

In some countries chemotherapeutic antibiotics have been banned for this purpose where they are also used in human or veterinary therapy purposes. Up until 1969 (Anon, 1969) low levels of chemotherapeutic antibiotics, in addition to copper sulphate, were used as growth promoting agents in Europe. 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 (with the exception of zinc bacitracin which, at the time, was used purely topically in human medicine) was firstly banned in the UK. This led to similar bans in the EU but this has not so far happened in the United States or many other countries. After 1969 the pharmaceutical companies developed new antibiotics, which were growth promoting but had no direct effect on *Salmonella* or *E. coli*. Their spectrum of activity was different. However, because they affected members of the gut flora, which were themselves inhibitory to *Salmonella* colonisation, they altered the susceptibility of poultry to infection with *Salmonella*. Some, such as tylosin and nitrovin and the glycopeptide, avoparcin considerably increased faecal excretion by *Salmonella* (Smith and Tucker, 1980b; Smith *et al.*, 1985). Using lower concentrations in the feed can reduce this effect. In addition to the effect on *Salmonella*, avoparcin also selects for resistance to glycopeptides, such as vancomycin in *Enterococcus faecium* (Wegener *et al.*, 1999). This is potentially a very great public health threat since the transposon can transfer the resistance to multiit-resistant strains of *Staphylococcus aureus* (Noble *et al.*, 1992). In addition and since poultry and pig *E. faecium* strains can colonise the human gut (Berchieri, 1999), the development of vancomycin resistant *S. aureus* concerns those involved in human public health. The use of growth promoting antibiotics,

including avoparcin, has now been banned in the EU. This sort of action, without controlling import of poultry and poultry meat from outside the trading bloc, does not, however, completely solve the problem.

Role of 'competitive exclusion'

Because young poultry, reared intensively, are slow to develop the complex intestinal microflora of older birds, they are particularly prone to colonisation with food-poisoning salmonellas. Colonisation resistance can be markedly increased, however, by the early establishment of an adult-type flora through oral administration of the requisite organisms. The phenomenon in poultry was first demonstrated by Nurmi and Rantala (1973) and is usually termed 'competitive exclusion' (CE). Protection develops rapidly, is apparently unaffected by the breed, sex or immune status of recipient birds and is active against all host non-specific serotypes studied so far (Mead, 2000). Commercial CE products contain a wide variety of viable bacteria that are provided by cultures of caecal material from selected donor birds. Their exact composition is unknown and such products must be extensively tested to ensure the absence of all known avian and human pathogens, with no demonstrable hazard to either users or recipients of the material. Sweden is one country with a relatively long history of using CE treatment without any evidence of adverse effects (Wierup *et al.*, 1992). Although it is recognised that a treatment product of fully defined composition would be more desirable in relation to safety and quality control, experience suggests that defined preparations are less protective and tend to lose potency during subculture and storage (Stavric and D'Aoust, 1993).

Prophylactic use in newly hatched chicks is one application of CE treatment. Another is to use it following antibiotic therapy for a disease condition in the birds or to eliminate an existing *Salmonella* infection. The purpose in this case is to repair any damage to the gut flora that may have been caused by the medication. The combined treatment is permitted in some countries where there would otherwise be a legal requirement to slaughter breeder flocks infected with *Salmonella enteritidis* or *S. typhimurium*. A third type of situation is one in which the birds have been stressed by poor handling or management practices that could disrupt the normal gut flora; however, this application has been little studied as yet. Whatever the situation, CE treatment should be given promptly and for prophylactic use it is best administered in the hatchery by automated spraying of coarse droplets in the bird dispatch area (Schneitz, 1992). Treatment of older birds is usually via the drinking water. CE preparations of chicken origin can also protect turkey poults against salmonellas (Impey *et al.*, 1982), thus indicating a high degree of compatibility between the floras of these two avian species.

Many laboratory-scale studies in different countries have confirmed the efficacy of CE treatment in controlling salmonella colonisation, although for unknown reasons, protection is rarely complete (Mead, 2000). In treated flocks, the proportion of birds becoming *Salmonella*-positive is reduced in comparison with untreated controls and for those birds that do become carriers, the numbers of salmonellas being shed are also generally lower. Protection of chicks tends to be greatest at the lower levels of *Salmonella* challenge. Unlike the conditions of laboratory studies, there are many potential variables in field trials and, because the *Salmonella* challenge cannot be predicted with certainty, extensive replication is needed to obtain meaningful results. Early experience of CE treatment in the field was disappointing (Pivnick and Nurmi, 1982) and it appears that effectiveness depends upon a supply of *Salmonella*-free chicks from the hatchery. For example, in the study of Deruyttere *et al.*, (1997), involving a million broilers, 24% of untreated, control flocks became *Salmonella*-positive, but none of the flocks given a CE

preparation. In this study, only chicks from *Salmonella*-free parent stock were used. Other studies have also indicated a positive effect of using such products in the field (Palmu and Camelin, 1997; Barbour *et al.*, 1999).

Under experimental conditions, it has been shown that CE treatment has a bacteriostatic effect on a subsequent *Salmonella* challenge, thus preventing colonisation (Impey and Mead, 1989). However, the exact mechanism of the inhibitory effect is unknown. The fact that protection of chicks first starts to become apparent within only a few hours of treatment is consistent with the view that competition between salmonellas and the flora for receptor sites in the gut is a key feature. Also, there are known to be inhibitory concentrations of certain short-chain fatty acids, which are metabolic end products of anaerobes and are most active in the undissociated state. Other possible factors include competition for limiting nutrients in the gut, conditions of low redox potential and production of H_2S and bacteriocins by elements of the flora, but their role in limiting salmonella colonisation is uncertain.

On its own, CE treatment is not a panacea and its effectiveness under commercial conditions depends upon being part of an overall *Salmonella* control programme. The treatment has the advantage of being easily applied at relatively low cost and it is compatible with other on-farm control measures, such as vaccination of the birds and treatment of feed with short-chain fatty acids. Preparations used for the purpose are derived from the 'normal' caecal microflora of healthy, adult birds that have been shown to be free from various pathogens. Therefore, the component organisms represent those that eventually would be acquired naturally by the birds, and, as well as controlling salmonellas, they appear to be active against other pathogens, including strains of *Escherichia coli* that are pathogenic for poultry and humans (Weinack *et al.*, 1981; Stavric *et al.*, 1992; Hakkinen and Schneitz, 1996) and *Clostridium perfringens* (Snoeyenbos *et al.*, 1983; Elwinger *et al.*, 1992). The latter organism is a cause of necrotic enteritis in poultry and is currently associated with reduced usage of antimicrobial feed additives within the Industry.

Salmonella vaccines for poultry

Between 1985 and 1990, the prevalence of *S. enteritidis* infection increased markedly in man and this was associated with the consumption of table eggs; more recently concern has occurred because multiple resistant strains of *S. typhimurium* DT104 have been isolated from poultry. Widespread use of antibiotics has been one of the factors associated with an increased prevalence of antibiotic resistant *Salmonella* in the UK, which has led to government enquiries both at home and abroad. One of the recommendations has been that vaccines should be developed for the control of *Salmonella* infection in farm animals. With that in mind, factors which may affect the efficacy and use of *Salmonella* vaccines in poultry and future developments are of significance. The host-adapted *Salmonella* serovars *S. pullorum* and *S. gallinarum* are important causes of disease in poultry but of no public health significance. Other serovars however, may cause little in the way of disease, except in young chicks, but are of public health importance and vaccination of poultry in these circumstances will be driven more by public health considerations rather than from expectations of securing marked improvements in production efficiency.

In 1886, E. Salmon and T. Smith showed that pigeons given heat-killed *Salmonella* could be protected against subsequent *Salmonella* challenge. This served as a model for later human typhoid vaccines whose use remained controversial until adequate field trials were done in the 1960s. *Salmonella* vaccines have been used in several species of domestic animals but in many instances it has been difficult to assess their efficacy because properly controlled field trials have not been done.

The requirements of an ideal *Salmonella* vaccine are that it should:

- Prevent disease caused by the prevalent *Salmonella* serovar and related serovars
- Minimise the duration and extent of *Salmonella* excretion and not lead to the development of carriers
- Be devoid of undesirable side effects
- Stimulate immunity which is transferred from the parent to the offspring
- Stimulate active immunity rapidly even in the presence of passive immunity
- Be easy to administer

And in the case of live vaccines:

- Should be stable and non-reverting and be distinguishable from wild type strains and should not readily survive in the environment

KILLED VACCINES

Heat inactivation has been used for many years with conflicting results but it is possible that those reported in the literature may have arisen from the inadvertent destruction of labile antigens during the preparation of the vaccine. *S. enteritidis* grown under conditions of iron restriction (Salenvac, Intervet), to simulate conditions in the host and to produce *in-vivo* antigens, has been used to prepare an inactivated vaccine which has been licensed in a number of European countries and is now in widespread use. Field trials in the UK showed that the vaccine decreased *Salmonella* shedding and increased productivity of broiler breeders (Feberwee *et al.*, 2000). Another strategy has been to use potent oil-adjuvants vaccines against *S. enteritidis* which are available in the USA and some European countries *e.g.* Talovac 109SE in Germany.

LIVE VACCINES

A live semi-rough strain of *S. gallinarum* (9R) has been used for many years for prevention and control of Fowl Typhoid. This vaccine was developed by empirical means and the reasons for its avirulence are unknown. It has also been used in the prevention of *S. enteritidis* infection in chickens (Witvliet *et al.*, 1997; Feberwee *et al.*, 2001).

Many other live, attenuated *Salmonella* vaccines, which include cell wall deficient strains, *e.g. galE* mutants, deletion mutants in various genes *e.g. aro* -, and other auxotrophic mutants, have been tested experimentally in poultry (Curtiss *et al.*, 1991; Cooper *et al.*, 1994) but only a few are available commercially. Commercially available *S. typhimurium* vaccines are:

a) Deletion mutations in the genes for adenylate cyclase and cAMP receptor proteins which render the serovar avirulent but immunogenic (Curtiss *et al.*, 1991), (MeganVac1, USA). However, the mutants are still able to induce gastro-enteritis (Barrow *et al.*, 2001).
b) Mutants based on adenine and histidine auxotrophy (Zoosaloral, H Dessau-Tornau GmbH)
c) Metabolic drift mutations, with resistance to nalidixic acid and rifampicin affecting the gyrase and RNA-polymerase and additional sensitivity to surfactants and anionic tensides that reduce environmental survival (Salmonella VacT: TAD).

Some live vaccines, if administered orally, are able to induce a CE-like exclusion effect on other *Salmonella* strains (Martin *et al.*, 1996).

EFFICACY OF *SALMONELLA* vaccines

Significant protection of vaccinated poultry from *Salmonella* infection has been demonstrated in many challenge experiments. The degree of protection is influenced by the vaccine type and the route of administration. Live vaccines have usually been administered orally, but this may require higher doses administered on a number of occasions.

Parenteral administration has been shown to reduce the severity of clinical signs and to protect against *Salmonella* invasion. While immunisation is effective in reducing the duration and numbers of *Salmonella* shed by infected animals, excretion is not eliminated completely.

Passive immunity in chickens occurs through the transfer of antibody to the egg, but there have been few studies of its effect on *Salmonella* colonisation in the newly hatched chicken or the likely effect on the use of live oral *Salmonella* vaccines. Hassan and Curtiss (1996) found reduced *Salmonella* colonisation in the progeny on immunised hens, but the maternal antibody reduced the efficacy of oral immunisation.

One problem area in the use of vaccines to control paratyphoid infections in poultry is whether vaccines prepared with one serovar, *e.g.* Typhimurium, will give protection against other serovars, especially those belonging to different serogroups *e.g.* enteritidis. Experimental results have been equivocal and since a wide range of different serovars have been isolated from poultry it would suggest that future vaccines will need to be include the predominant serovars in the vaccine preparation. Another problem area is that vaccination will result in the production of antibodies by the immunised birds, which may be indistinguishable from those that occur after natural infection and thus interfere with the use of serological tests for diagnosis.

FIELD TRIALS

These have been difficult to do, because it is usually not possible to have immunised and non-immunised groups of birds on the same farm. Likewise large numbers of birds are necessary to achieve statistically valid results. During the evaluation of an inactivated *S. enteritidis* vaccine over two million birds were vaccinated. A study in Germany, found that the use of vaccines in 600,000 birds on 16 farms reduced the *S. enteritidis* infection rate from 10% to 2.5% (Vielitz *et al.*, 1996). A recent field trial in the Netherlands (Feberwee *et al.*, 2001) comprised 64 vaccinated flocks (15 vaccine A, 49 vaccine B) and 608 non-vaccinated flocks, Even so interpretation of results was difficult, with no significant results for vaccine A because of the small number of flocks and in the case of the inactivated vaccine B it was concluded that it contributed to a reduction of *S. enteritidis* reinfection in the flocks.

In the UK, many breeder and layer flocks are now vaccinated against *S. enteritidis*, which has led to a reduced prevalence of the serovar in poultry.

Field trials have shown that both inactivated and live vaccines have been effective in reducing *Salmonella* shedding, which in turn reduces environmental contamination and the risk of horizontal transmission. The use of *Salmonella* vaccines has also been shown to increase poultry productivity in terms of egg production and hatchability. *Salmonella* vaccines have an important role to play in poultry production, but their use must be part of an overall strategy which includes improvements in husbandry, hygiene and biosecurity.

Genetic resistance

Variation between chicken lines in susceptibility to *Salmonella* infection, suggesting a host genetic component has led to many studies with a view to exploitation. A variety of infection models have been used, including acute systemic disease, intestinal carriage and contamination of yolks and other internal organs.

Resistance to systemic disease, assessed by the mortality post inoculation, is not expressed during the invasive phase of infection but is thought to be an attribute of the macrophage (Kramer *et al.*,1999) and was found to be correlated with the magnitude of the oxidative burst generated within the macrophage during infection (Wigley *et al.*,

2002). Resistance to a variety of serotypes including *S. Pullorum*, *S. gallinarum*, *S. typhimurium* and *S. enteritidis* has been demonstrated in both outbred and inbred lines (Bumstead and Barrow, 1993; Guillot *et al.*, 1995 ; Gast and Benson, 1995). Comparison of the same lines shows similar patterns of susceptibility and resistance to those serotypes and, not surprisingly, to various phage types of *S. enteritidis* (Gast and Benson, 1995), suggesting a common mechanism.

Studies of the Mendelian inheritance of resistance (Bumstead and Barrow, 1988) revealed the resistance to mortality induced by *S. typhimurium* to be transmitted in a dominant manner. The pattern of inheritance was compatible with the involvement of a single autosomal gene or of a group of tightly linked genes. A new gene, *SAL1*, appears to be responsible for up to 50% of the inherited resistance (Mariani *et al.*, 2001). However, other genes also contribute. Two homologues of genes controlling *Salmonella* resistance in mouse, *Nramp1* (Vidal *et al.*, 1993) and *TLR4*, previously named *Lps* (O'Brien, *et al.*, 1980), are major candidates and have been tested by Hu *et al.*, (1997). The chicken homologue of *NRAMP1* has been cloned and has been tested directly, in contrast *TLR4*, which was tested using the marker gene tenascin C (*TNC*) as a probe. Both *NRAMP1* and *TNC* genes contributed significantly to survival in *S. typhimurium* infection during the first 7 days pi. Together, *NRAMP1* and *TNC* explained 33% of the early differences in resistance to infection in susceptible and resistant lines. The association with the Major Histocompatibility Complex (MHC) is less clear since Cotter *et al.*, (1998) have suggested a contribution whereas Bumstead and Barrow (1988) found no association in their model after typing the challenged birds for MHC by serological tests. MHC is more likely to be involved during the later stages of infection.

Studies with outbred lines have estimated heritability of mortality, intestinal colonisation and splenic infection (Berthelot *et al.*, 1998 ; Janss and Bolder, 2000). These predicted that selection of divergent lines for increased and decreased resistance is practicable. However, this idea must be considered with care, as comparison of genetic parameters of 3 traits for *Salmonella* resistance, i.e. mortality, survival time and caecal carriage, indicated that selection on decreased mortality could be unfavourable for faecal shedding (Janss and Bolder, 2000). However, these studies were carried out in the absence of any information on the gene(s) involved in each phenotype.

Resistance to intestinal carriage in young (Duchet-Suchaux *et al.*, 1997) and to contamination of yolks, other internal organs and intestine in adult has been observed with serotype *S. enteritidis* in outbred lines (Guillot *et al.*, 1995 ; Duchet-Suchaux *et al.*, 1997). The biological basis of this is currently poorly understood as is its genetic basis, except that it is thought to be autosomal (Duchet-Suchaux *et al.*, 1997; Berthelot *et al.*, 1998). Genes associated with resistance to systemic disease are unlikely to be involved in a major way.

Once genes have been identified considerable potential will exist for their selection as a means of increasing resistance to *Salmonella*, either in its own right or in combination with other control measures, including others mentioned above.

Conclusion

There are a number of merits in the inclusion of a biological control-component in a comprehensive control regimen. However, as has been emphasised many times in the past, it is necessary to maintain strict attention to hygiene and management and not simply rely on the biological aspects of control.

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