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**EPIDEMIOLOGICAL STUDIES OF SALMONELLA AND
CAMPYLOBACTER IN POULTRY**

- 1 A case control study of *Salmonella enteritidis* infection in
British poultry breeding flocks**
- 2 Epidemiological studies of thermophilic campylobacter
infection in British broiler flocks**

A thesis presented for the degree of Doctor of Philosophy
at
The London School of Hygiene and Tropical Medicine
Keppel Street, London
(University of London)

Sarah Jane Evans

1997

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Abstract

Salmonella and *campylobacter* are major human bacterial enteropathogens and the reported incidence is increasing in Great Britain despite efforts to control the problem. Most cases of disease are acquired by the ingestion of contaminated food and poultry are primary sources of infection. It is essential to reduce the carriage of these bacteria by poultry and the work contained within this thesis contributes to the understanding of the epidemiology of these infections in British poultry flocks. This knowledge is fundamental to the development of appropriate preventive measures.

A national case control study of *Salmonella enteritidis* PT4 infection in poultry breeding flocks identified major risk factors for infection. Both the feed and the farm environment, including animal reservoirs, were shown to be sources of infection but there was no evidence of vertical transmission of infection from grandparent flocks. The most important factors that are likely to reduce the risk of infection in breeding flocks are thorough cleansing and disinfection of sites following a salmonella incident, strict hygiene measures between the site and the hatchery, effective isolation of the poultry unit from other domestic species and the use of heat treated poultry feed.

Epidemiological studies of *campylobacter* infection in broiler flocks revealed that *Campylobacter jejuni* infection was widespread within the British broiler industry. The national prevalence survey reported that at 5 weeks of age 45% of flocks were colonised (95% confidence limits: 36.9-53.1%) and a longitudinal study showed that this increased to more than 90% by 7 weeks of age. The environment, including contaminated drinking systems, appeared to be the main reservoir of *campylobacter* infection for broiler flocks and the use of strict hygiene barriers at the entrances to broiler houses was shown to reduce the risk of infection. Interventions aimed at improving hygiene standards on sites are thus likely to reduce the prevalence of *campylobacter* infection at slaughter.

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Preface

Since qualifying as a veterinary surgeon in 1987, I have been particularly interested in public health issues and zoonoses. I was most fortunate to be able to develop this interest further when I joined the Epidemiology Department of the Central Veterinary Laboratory in 1991. Since then I have worked within the area of food-borne zoonoses and my fascination with the subject has been maintained.

I am fortunate to have worked with a number of eminent epidemiologists and I would particularly like to express my thanks to my supervisors Laura Rodrigues and John Wilesmith who have supported me throughout my studies. I would also like to thank other members of the Central Veterinary Laboratory who have helped make these projects a success. In particular, I would like to thank Robin Sayers for his statistical assistance and Diane Newell for bacteriological support. Finally none of these projects would have been possible without the support of the British poultry industry and the veterinary staff of MAFF and SOAFD who were involved in the data collection for the *Salmonella enteritidis* case control study.

On a personal note, I acknowledge the support and patience of my family and I would like to dedicate this work to my father, Tom Voysey, who sadly died last year.

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2 April 1997

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CHAPTER 1

Introduction

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Introduction

Human illness caused by the consumption or handling of foods contaminated by micro-organisms (food-borne disease) imposes a considerable economic burden on society. Costs to the health sector arise from the surveillance, investigation, treatment and control of these diseases and production losses due to absence from work are borne by industry. The food industry incurs costs in reducing the risk of infection and, if implicated in outbreaks of disease, there can be substantial costs from loss of business, productivity and goodwill. Intangible costs arise from human pain and suffering caused by these unpleasant diseases and the value of lost lives.

Despite considerable efforts to control micro-organisms in foods and strict food hygiene regulations the incidence of food-borne illness in Great Britain and other developed countries is increasing. The reasons for the increase are unclear, although there are a number of possible contributing factors:-

- 1 Better surveillance and increased level of awareness, improved microbiological methods and the identification of new pathogens eg. *Escherichia coli* 0157;
- 2 Changing population sensitivities - aging populations, immuno-deficiency;
- 3 Changes in food marketing and eating habits - centralised food processing, reduced usage of preservatives, increased consumption of fresh foods, pre-prepared foods and foods consumed outside the home;
- 4 Diminished food preparation skills;
- 5 Intensification of farming systems favouring spread of these infections;
- 6 Increased global trade in food;
- 7 Increased international travel.

Contaminated poultry products are a major source of food-borne disease and the large rise in poultry consumption since the 1950's, due to the reduction in cost of poultry meat through improvements in farming methods, may also have contributed to the rise in food-borne illness. Although several pathogens have been associated with poultry products, salmonellosis and campylobacteriosis are of primary concern in Great Britain and many other developed countries. This thesis focuses on these important infections of poultry and the associated risk of human food-borne illness.

Epidemiological Feature	Salmonella	Campylobacter
Laboratory reports (GB 1995)	32,824	48,279
Most common serotype	<i>S. enteritidis</i>	<i>C. jejuni</i>
Seasonality	Reports peak in summer months	Reports peak in late spring and autumn
Clinical signs	Diarrhoea & vomiting, fever, headache	Profuse diarrhoea, may be blood stained, rarely vomiting, sometimes fever
Infectious dose	Usually $>10^5$ cells	< 500 cells
Incubation period	12-48 hours	2-5 days
Duration of symptoms	1-3 days	1-7 days
Pattern of disease	2/3 cases sporadic, remainder outbreaks	Vast majority of cases sporadic, outbreaks rare
Sources of infection (sporadic cases)	Raw meat, especially poultry eggs	Raw meat, especially poultry pets (cats, dogs)
Sources of infection (outbreaks)	Raw milk eggs	Raw milk contaminated water
Person to person transmission	Occasional, mainly in institutions	Very rare
Microbial growth in foods	Yes	No

Figure 1.1. Comparison of the epidemiological features of human disease caused by salmonella and campylobacter in Great Britain

Food-borne disease in man caused by salmonella and campylobacter infection

The main epidemiological features of human disease caused by salmonella and campylobacter in Great Britain are compared in figure 1.1 and discussed in detail below.

Salmonellosis

Incidence

Many different salmonella serotypes can cause human salmonellosis but *S. enteritidis* and *S. typhimurium* currently account for over 75% of the laboratory reports of salmonella to the Public Health Laboratory Service Communicable Disease Surveillance Centre (PHLS-CDSC) from England and Wales and the Scottish Centre for Infection and Environmental Health (SCIEH) (HMSO 1995). There has been a huge rise in reports since the mid 1980's in Great Britain. In 1981 there were less than 13,000 reports of salmonella and just over 10% of these were of the serotype *enteritidis* and a third of the isolates of this serotype were of a single subtype known as phage type 4. Annual rates of salmonellosis doubled between 1981 and 1987 but during the corresponding period there was a twelve fold increase in the rate of *S. enteritidis* PT4, which more than doubled again between 1987 and 1989 to a peak rate of more than 30/100,000 population. Laboratory reports only represent the tip of the iceberg as not all cases seek medical attention or are microbiologically confirmed. It has been estimated that the number of salmonella reports represent between 1% and 10% of all cases (HMSO 1993, Roberts and Sockett 1994). The rate of *S. enteritidis* PT4 remained high until 1994 when the first reduction in the number of reports since the start of the epidemic was evident. During 1994, there were 33,500 salmonella reports; 57% were *S. enteritidis* and of these *S. enteritidis* PT4 accounted for 79%. There were 20% fewer reports of *S. enteritidis* PT4 in 1994 than in the previous year and this trend continued in 1995. The number of reports of infections due to other salmonella serotypes has remained relatively unchanged in the last 15 years (figures 1.2 and 1.3). There is a distinct seasonality of infection. In temperate countries, the number of reports increase with the onset of warmer temperatures in May and June and decline in the Autumn.

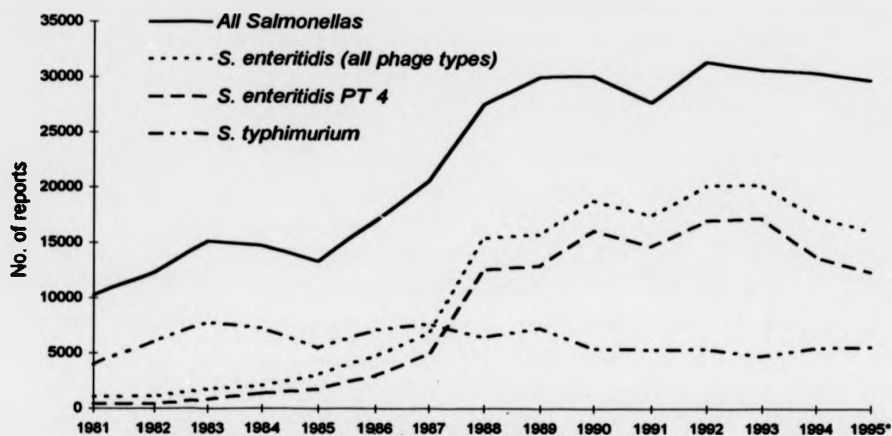


Figure 1.2. Laboratory reports of faecal isolates of salmonella in England and Wales (1981 - 1995)

*Provisional figures

Source: PHLS - CDSC

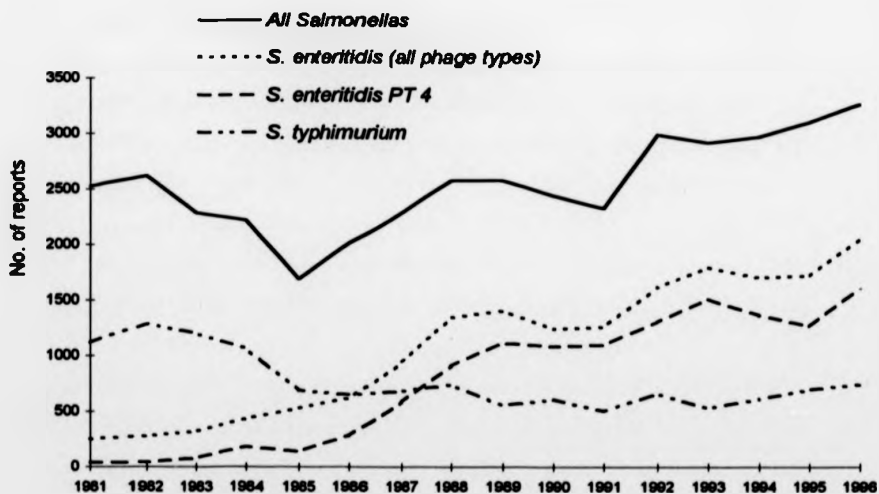


Figure 1.3. Laboratory reports of faecal isolates of salmonella in Scotland (1981 - 1996)

Source: SCIEH

Similar epidemics of *S. enteritidis* have been observed in other European countries, the United States of America and other areas of the world although the predominant phage types involved differ between countries, indicating that a single source for the global increase is unlikely (Rodrigue and others 1990). Phage type 4 is most common in Great Britain, whereas phage type 8 and 13A predominate in the United States of America. However phage conversion, particularly between phage type 4 and 8, is known to occur (Rankin and Platt 1995). The reason for the *S. enteritidis* pandemic is unknown. There is no evidence to suggest any change in the bacterium that may account for the epidemic (Cox 1995). There have been previous epidemics of other salmonella serotypes and the observed pattern is one of an epidemic rise of one serotype which remains common for many years but is eventually superseded by another serotype. It has been proposed that this phenomenon is related to the prevalence of these organisms in foods at the time (HMSO 1996).

Clinical signs

Salmonellosis in man usually causes fever, abdominal pain, vomiting and diarrhoea which persists for a few days and can be severe, occasionally resulting in death, but infection can also be asymptomatic. Young children, the elderly and the immunocompromised are particularly susceptible. Multi-person outbreaks of infection are more common than with campylobacter.

Sources of infection

Salmonellas are ubiquitous and can be isolated from the gut contents of a wide range of animal species. Transmission to man is usually food-borne from eating raw or undercooked meat, milk or eggs or by cross-contamination to other foods in the kitchen which are eaten without further cooking. Salmonellas grow rapidly in foods at room temperature and will survive refrigeration and freezing but are killed by heat over 60°C. Person to person spread is most common in institutions but accounts for less than 10% of cases.

Poultry is thought to be a major source of human salmonellosis and is particularly associated with *S. enteritidis* infections (HMSO 1996). Poultry meat is frequently

contaminated with salmonella (Humphrey and others 1988, Rampling and others 1989) but early in the *S. enteritidis* epidemic it emerged that eggs were also an important source of this serotype (PHLS 1989). This is due to the ability of *S. enteritidis* infected laying flocks to produce infected eggs. The investigation of reported outbreaks of infection implicated eggs or egg products as more frequent sources of infection than poultry meat, particularly in the early years of the epidemic (St Louis and others 1988, Telzak and others 1990, Mishu and others 1991, Sockett and others 1993). However, outbreaks account for less than a third of cases and the majority of cases are sporadic (Duguid and North 1991, Bogel and others 1995). The few case control studies that have been conducted of sporadic cases of human infection with *S. enteritidis* identified both eggs and poultry meat as sources of infection (Coyle and others 1988, Cowden and others 1989, Hedberg and others 1993).

Surveys conducted by the PHLS of English retail premises suggest that at the peak of the epidemic approximately 40-60% of fresh and frozen chicken carcasses were contaminated with salmonella and *S. enteritidis* PT4 was the most common type found (PHLS 1989, Roberts 1991). Similar surveys of retail eggs suggested 0.9% were contaminated with salmonella, again predominantly *S. enteritidis* PT4 (de Louvois 1993). However, a survey of retail United Kingdom produced chickens in the winter and spring of 1993/1994 showed that the level of salmonella contamination had decreased to 33% of fresh and 41% of frozen chickens sampled and *S. enteritidis* PT4 was isolated from 16% of all chickens sampled (HMSO 1996). In contrast with campylobacter contamination of chicken meat, the number of salmonellas present on poultry products are extremely low and bacterial multiplication may be required to cause disease.

Economic cost

The tangible costs of reported and unreported human salmonella infection in England and Wales in 1992 were calculated to lie between £350 and £502 million, of which, £224-321 million were attributed to infection with *S. enteritidis* (Roberts and Sockett 1994).

Campylobacteriosis

Incidence

Thermophilic campylobacters have become the most frequently implicated infectious cause of human gastro-enteritis in Great Britain. The number of reports have exceeded those of salmonella since 1981 in England and Wales and since 1985 in Scotland. Of the three main species of thermophilic campylobacter, *C. jejuni* is by far the most important and is isolated from 98% of cases. Precise measures of the incidence are not available as many cases are unreported. However, laboratory reports to the PHLS gave an annual reported incidence of 68/100,000 in 1990 (Pearson and Healing 1992) and a survey conducted in a General Practice calculated an annual incidence of 11/1000 (Kendall and Tanner 1982), which is similar to that calculated for the United States of America (Skirrow and Blaser 1992). The number of reports of campylobacter infection have increased dramatically in recent years (figure 1.4). In England and Wales the number of reports increased from 12,000 in 1981 to 44,000 in 1995. A similar, but less striking, picture has been observed in Scotland. Highest rates of infection are in children and young adults, particularly males. The number of reports peak in May or June, 6-8 weeks before the peak in salmonella cases, and then decline to less than 50% of this level in the winter months.

Clinical signs

Most cases of campylobacteriosis are sporadic and large outbreaks of illness are relatively rare. Illness occurs 2-10 days after exposure and the presenting symptoms are diarrhoea, which may be blood-stained, abdominal pain and sometimes fever. The disease is usually self-limiting, lasting about 5 days. Complications are uncommon but can be serious. Recently, *C. jejuni* has been associated with Guillain-Barré syndrome which is a post-infectious neurological disorder and can be fatal. Asymptomatic infection in developed countries is rare as is person to person transmission. In contrast, in developing countries where campylobacteriosis is hyper-endemic, clinical illness in adults is rare due to the early acquisition of immunity by children persistently exposed to multiple strains of infection. Some individuals may also have a degree of acquired immunity to campylobacter in developed countries (eg. farmers, slaughterhouse workers, butchers).

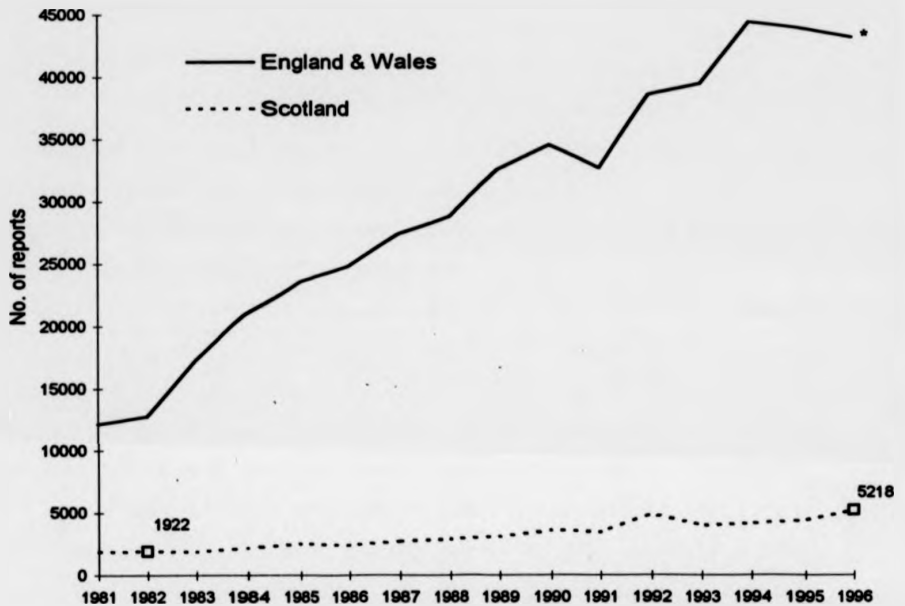


Figure 1.4. Laboratory reports of faecal isolates of campylobacter in England, Wales and Scotland (1981 - 1996)

*Provisional data

Source: PHLS - CDSC, SCIEH

Sources of infection

The precise role that infected animals and birds play in the human disease is not clearly defined. However, the majority of infections are food-borne and evidence has been accumulating that poultry are the major source of infection. Several studies have found high *C. jejuni* isolation rates in broiler farms and poultry processing plants (Prescott and Munroe 1982, Tauxe and others 1985, Hood and others 1988). Serotyping has revealed similar strains in poultry and man (Juven and Rogol 1986, Annan-Prah and Janc 1988) and case control studies in the human population have attributed at least half of all cases to the consumption or handling of chicken (Harris and others 1986, Deming and others 1987). However, a case control study in England and Wales found that the handling and consumption of whole chicken in the

home significantly reduced the risk of campylobacteriosis (Adak and others 1995). Despite the small size of this study, it indicated that poultry meat may not always be a risk factor for campylobacter infection. The authors concluded that immunity was playing a contributing role in the epidemiology of infection.

Campylobacters are fragile organisms and are susceptible to most methods commonly used to eliminate enteropathogens from foods. However, the incidence of human campylobacteriosis is high. This may be due to a combination of factors including the high numbers of organisms present on raw chicken, the low infective dose and the ease with which cross-contamination can occur during food preparation to utensils or foods which are not subsequently cooked. It has been shown that a small drop of raw chicken juice can be sufficient to provide the infective dose for man.

There are other less important but well-recognised sources of campylobacter infection for man. These include red meats (Fricker and Park 1989), unpasteurised milk or contamination of milk delivered to the doorstep by wild birds pecking through the bottle tops (Hudson and others 1990, Riordan and others 1993), contaminated drinking or recreational water (Mentzing 1981), direct contact with infected animals especially domestic pets (Skirrow 1981, Miller and others 1987) and at least 10% of cases in Great Britain are attributed to foreign travel.

Economic cost

Limited studies on the economic costs associated with campylobacter infection suggest that costs may be similar to those for salmonella (Socokett and Pearson 1988).

Structure of the British poultry industry

Poultry species include chickens, turkeys, ducks and geese but, in the context of this thesis, poultry is considered to be synonymous with chicken. Chickens are responsible for nearly all table egg and more than 80% of poultry meat production in Great Britain. Food-borne diseases carried by other species of poultry are likely to play a much smaller role in human disease than those carried by chickens.

The poultry industry is one of the most advanced sectors of agriculture in Great Britain. The intensification of farming methods over the last 40 years has resulted in a high annual output of low cost chicken meat and table eggs. The vast majority of broiler chicks are reared within controlled ventilation broiler houses and 90% of eggs for human consumption are laid by hens kept in battery cages.

The two sectors of the industry are clearly separated and stock is bred for either eating or laying purposes. However, a similar breeding and production chain exists within the meat and egg sectors (figure 1.5). Elite and great grandparent breeding flocks are bred for genetic characteristics. Grandparent flocks are bred from the best of the elite lines and parent stock are produced from these birds. Parent breeding flocks produce fertile eggs from which broiler or layer chicks are hatched. These chicks are then reared on specialised production farms.

There has been a marked trend towards centralised production and both sectors of the industry are now in the control of relatively few companies. Seventy per cent of table eggs are produced by less than 300 of Britains' 26,000 laying bird holdings and only 8 companies control over 75% of the broiler chicken market (HMSO 1996). Companies tend to be fully integrated in that they have parent flocks, hatcheries, production farms, feed mills and egg packing or processing plants within the same management group.

Currently, in Great Britain, 6.5 million broiler breeding parent birds produce 700 million broiler chicks and 33 million laying hens produce 835 million dozen eggs per annum. The United Kingdom produces 977,000 tonnes of chicken meat, of which 107,000 tonnes are exported. In addition, 200,000 tonnes of chicken meat are imported, mainly from France. Since 1989, poultry meat has been the most popular type of meat consumed within the United Kingdom (accounting for 37% of the primary meat market in 1995). Fifty seven per cent of chicken meat is sold fresh, 38% frozen and 5% cooked. There is an extremely wide product range including whole birds, portions and further processed or cooked products. In recent years there has been a large increase in sales of added value products such as ready meals, which

PRODUCTION CHAINS

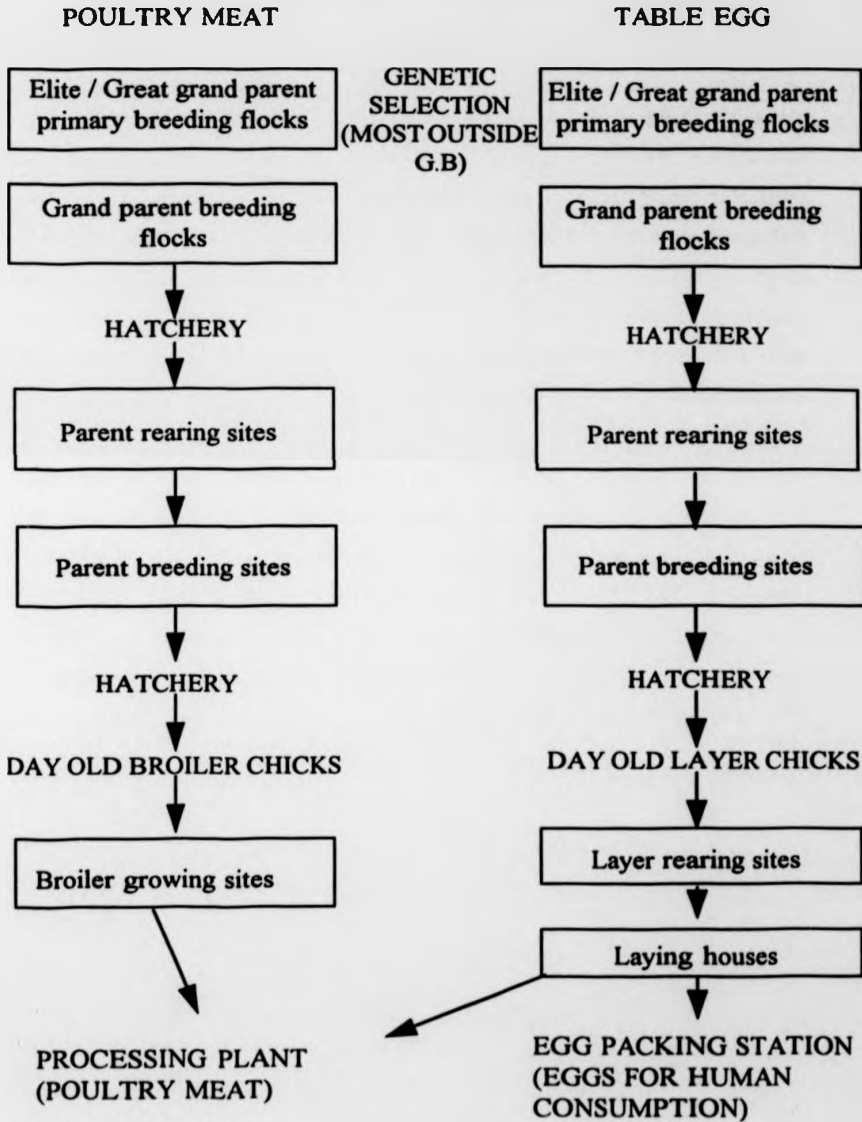


Figure 1.5. Structure of the British Poultry Industry

in 1995 represented 35% of all chicken sales. The retail chicken market was worth over £1.3 billion in 1995 and the gross output of the poultry industry is about 10% of national agricultural production (British Chicken Information Service 1996).

Aims of studies

It is evident that the control of food-borne illness due to infection with salmonella, particularly *S. enteritidis*, and campylobacter, mainly *C. jejuni*, is of great national and world wide importance. Poultry represent a major source of these pathogens and, in the absence of effective carcase decontamination methods and the failure of educational efforts to improve food handling and cooking practices, there is an immediate need for a cost-effective approach to reducing the prevalence of salmonella and campylobacter infection of poultry.

This thesis reports the results of a number of analytical epidemiological studies of these infections in commercial poultry flocks in Great Britain. Data collection occurred at a national level to ensure that the results were applicable to the national population. The primary aims of the studies were the elucidation of the epidemiology of salmonella and campylobacter infection in poultry in order to assist with the development of effective preventive measures to reduce the prevalence of infection with these organisms in the live bird.

**1 A CASE CONTROL STUDY OF
SALMONELLA ENTERITIDIS INFECTION
IN BRITISH POULTRY BREEDING
FLOCKS**

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CHAPTER 2

***Salmonella enteritidis* infection of poultry**

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Introduction

More than 2,000 different salmonella serovars have been described and they differ widely in their host range and pathogenicity. Infections are common in domestic poultry and, although many serovars have been identified, one serovar may be predominant for a number of years before being replaced by another. Since 1987, *Salmonella enteritidis* has been the most frequent serovar isolated from the national poultry flock (Great Britain). This increase was associated with the emergence of phage type 4. A similar situation has occurred in the rest of Europe and the Americas, although different phage types dominate in the United States of America and Canada (Rodrigue and others 1990).

S. enteritidis is well adapted to poultry and infection is not usually associated with clinical disease (Hopper and Mawer 1988, Humphrey and others 1989). In 1994, less than a quarter of isolations reported to MAFF were associated with clinical disease. However, *S. enteritidis* can cause systemic infection and morbidity rates ranging from 5 to 20%, with mortality rates of 6%, have been recorded during the first week of life (O'Brien 1988, McIlroy and others 1989). In affected chicks, the pathological findings include pericarditis, necrotic foci in the liver and indurated yolk sac remnants.

Adult chickens naturally infected with *S. enteritidis* are usually symptomless carriers of the bacteria, although the organism may be isolated from the ovaries and oviduct, liver, spleen and peritoneum (Hopper and Mawer 1988, Cooper and others 1989). Infected breeding and layer flocks usually show no decrease in egg production but some birds may become chronic carriers and excrete the organism intermittently (Williams 1972, Williams and Whitemore 1976).

Stresses such as food and water deprivation and intercurrent disease can increase the susceptibility of chickens to *S. enteritidis* (Holt 1993, Nakamura and others 1995) and also enhance the severity (Phillips and Opitz 1995), increase speed of transmission between birds (Holt 1995) or cause recrudescence of infection (Qin and others 1995). Chicken breeds have been found to vary in their susceptibility to salmonella

(Bumstead and Barrow 1993).

It has been shown that PT4 is more virulent and invasive in poultry than other phage types of *S. enteritidis* (Hinton and others 1990a, Barrow 1991). A gradation has also been found in the ability of strains of PT4 isolated in 1978, 1984 and 1988 to invade chicken livers suggesting an increase in the virulence of this strain during the period (Hinton and others 1990b).

Salmonella is a frequent food-borne infection of humans and large increases in the rate of human infection with *S. enteritidis* have occurred in parallel with the epidemic in poultry. Contaminated poultry products are a major source of human infection (Coyle and others 1988, Humphrey and others 1988, Cowden and others 1989, Roberts and Socket 1994) and, as with campylobacter, efforts to control salmonella in domestic poultry are mainly driven by public health implications.

Methods of detection

Salmonella can be isolated from bacteraemic birds by direct culture but the caecum is the most likely site for isolation in adult birds. Population screening methods must be capable of detecting low incidence infections of poultry, which are common, and methods have been developed to sample the environment as an indirect indicator of flock infection.

Various isolation methods are currently used and most involve a pre-enrichment step followed by selective enrichment in selenite, tetrathionate or Rapport-Vassiliadis medium and incubation at 37-42°C and the use of a selective plating media, such as MacConkey, deoxycholate citrate or brilliant green agar. Pre-enrichment in buffered peptone water, before selective enrichment in semi-solid media such as Diasalm has been shown to be the most sensitive method. Further subdivisions for epidemiological purposes can be achieved by phage typing schemes, plasmid profile analysis and other genetic techniques, biotyping and antimicrobial sensitivity testing. Threlfall and others (1994) detected 11 plasmid profile types within *S. enteritidis* PT4, however, the predominant type was detected in 70% of the isolates from

poultry, 92% from eggs and 90% from man.

A number of serological tests are available for the diagnosis of salmonella infection in poultry. The enzyme-linked immunosorbent assay (ELISA) is used in many countries for the identification of *S. enteritidis* infected flocks although bacteriological confirmation is recommended due to poor specificity. Two systems are currently used, the indirect ELISA and the competitive double antibody blocking ELISA, the former being favoured for monitoring purposes in Great Britain. A disadvantage of using diagnosis based on serology is that positive serology does not necessarily mean that the bird is currently infected and negative serology can be compatible with the early stages of infection prior to the development of an immune response. Interpretation of serological tests is further complicated by vaccination or antibiotic treatment of flocks.

Epidemiology of infection

Prevalence

Between 1981 and 1986 *S. enteritidis* accounted for 2.2% of the salmonella incidents recorded in chickens by the Ministry of Agriculture, Fisheries and Food (MAFF) but in 1987 the number of reports of this serotype tripled and the dramatic increase continued in 1988 by which stage *S. enteritidis* accounted for 50% of the salmonella reports in chickens. The number of incident reports continued to rise and peaked in 1990/1991 and then gradually declined during 1992/1993 followed by a more marked decline in recent years (figure 2.1). In 1989, in response to the epidemic, the British Government introduced a compulsory monitoring scheme for salmonella infection of poultry flocks. The increased level of monitoring may have resulted in an increase in the number of reports of salmonella from poultry and thus complicates the interpretation of trends over time, particularly in the early years of the epidemic. Throughout the epidemic the predominant strain of *S. enteritidis* was PT4 which has been isolated from more than three quarters of incidents (MAFF 1995). Surveys conducted by the PHLS of English retail premises suggest that at the peak of the epidemic 40-60% of fresh and frozen chickens were contaminated with salmonella and *S. enteritidis* PT4 was the most common type found (PHLS 1989, Roberts 1991). Similar

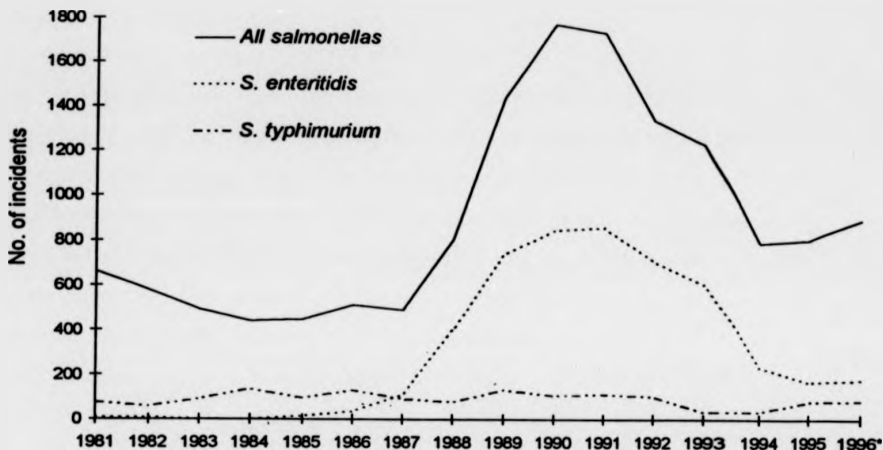


Figure 2.1. Salmonella incident reports in chickens in Great Britain (1981-1996)

*Provisional figures

Source: MAFF

surveys of retail eggs suggested 0.9% were contaminated with salmonella, again predominantly *S. enteritidis* PT4 (de Louvois 1993). However, a survey of retail raw chicken in the winter and spring of 1993/1994 showed that the level of salmonella contamination had decreased to 33% of fresh and 41% of frozen chickens sampled and *S. enteritidis* PT 4 was isolated from 16% of all chickens sampled (HMSO 1996).

Sources of infection

Vertical transmission

Breeding and production flocks from both the poultry meat and egg production sectors of the British poultry industry have been involved in the *S. enteritidis* epidemic. The hierarchical structure of the industry and the ability of *S. enteritidis* to be transmitted vertically to offspring via the egg may partly explain the widespread nature of the epidemic (O'Brien 1988, Lister 1988). There have been no confirmed flock infections with *S. enteritidis* in elite or grandparent breeding flocks since the start of the compulsory monitoring scheme for salmonella in 1989. However, *S. enteritidis*

PT4 was isolated from the ovules of two hens from a British grandparent flock, type unspecified, which was examined at depopulation in 1988 (O'Brien 1990). Anecdotal evidence from other countries also suggests that primary breeding flocks were infected in the late 1980's as *S. enteritidis* was isolated in Japan from broiler parent chicks which had been imported from England, and from a broiler grandparent flock in The Netherlands which had been hatched from eggs imported from the United Kingdom (Nakamura and others 1993, Edel 1994).

When a breeding flock is infected with *S. enteritidis*, a cycle can be established by which the organism passes via the eggs to the progeny. This cycle can occur by true ovarian transmission or, as is much more likely to happen, through faecal contamination of the egg surface. As the egg passes through the cloaca, salmonella in the faeces attach themselves to the warm, wet shell surface and may be drawn inside as it cools. Surface contamination may also occur in the nest boxes.

Hatcheries can serve as reservoirs of infection and cross-contamination in the hatching cabinets may dramatically increase the prevalence of salmonella infected chicks leaving the hatchery compared with the prevalence of infected eggs entering the hatchery (Bailey and others 1994).

Recent studies in The Netherlands concluded that vertical transmission of *S. enteritidis* to parent breeding flocks and to commercial laying flocks did not appear to be important, at least in recent years, as infections during the rearing period were rare (Fris and van den Bos 1995, van de Giessen and others 1994). The relative importance of vertical transmission as a route of *S. enteritidis* infection in British poultry flocks has not been established but is likely to vary by flock type, depending on the incidence of infection in the parent birds.

Feed contamination

Early speculation suggested that contaminated feed was involved in the epidemic but, although a recognised source of some salmonella serotypes (Jones and others 1989), there was controversy as to the importance of feed in the epidemiology of *S.*

enteritidis infection (HMSO 1992, Jones and Richardson 1996). The main reason for the controversy was the poor correlation between salmonella serotypes isolated from poultry and those found in feed. The incidence of salmonella contamination in feeds, detected by official MAFF testing of animal protein and finished feeds, is generally low but contamination rates were higher than current levels at the start of the epidemic. In 1989, 5% of home produced animal protein samples tested by MAFF were positive for salmonella but only 4 samples (7%) of these contained *S. enteritidis*. Since then the salmonella contamination rate of home produced animal protein has gradually decreased although the proportion of isolates found to be *S. enteritidis* has remained constant. Although salmonellae are isolated more frequently from consignments of imported animal protein, *S. enteritidis* is rarely found. In recent years, finished poultry feed samples have also been monitored but in 1993 and 1994 only 2.7% of samples tested were found to be contaminated with salmonella and less than 2% of these contained *S. enteritidis* (HMSO 1992, MAFF 1995). However, it has been observed that, due to the heterogeneity of infection in feed (Veldman and others 1995), the sensitivity of current monitoring procedures is poor and the ability of the organism to multiply from non-detectable numbers during improper feed storage has also been recognised (Davies 1992). It has been shown that it is possible to infect chicks with feed containing less than 1 salmonella per gram (Hinton 1988), but not every serotype or phage type has the same colonisation potential and poultry may become selectively colonized by the more virulent strains in feed, such as *S. enteritidis* PT4. A second reason for the disputed role of feed as a source of *S. enteritidis* was the limited epidemiological evidence from field investigations. There are, however, a number of studies that have identified poultry feed as a source of salmonellae. All breeder feed has been heat treated in Northern Ireland since early epidemiological investigations revealed that feed was a possible source of *S. enteritidis* for these flocks (McIlroy and others 1989). The levels of *S. enteritidis* in poultry in Northern Ireland have been lower than the rest of the United Kingdom but heat treatment of breeder feed was only one aspect of the comprehensive control policy adopted by the poultry industry. Humphrey and Lanning (1988) found that formic acid treatment of breeder feed significantly reduced the number of salmonella isolations from feed, litter, hatchery waste and chick box liners. Other recent studies

have implicated feed as a source of salmonella for poultry flocks although, as with the Humphrey and Lanning study, this risk may be associated with serotypes other than *S. enteritidis* (Henken and others 1992, Jacobs-Reitsma and others 1994, Angen and others 1996).

Role of wildlife

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

Although *S. enteritidis* infection in mice on poultry units was reported 15 years previously (Krabisch and Dorn 1980), their significance as vectors of *S. enteritidis* on poultry units has only received attention relatively recently (Opitz 1992, Henzler and Opitz 1992). Naturally infected mice, captured at depopulation of poultry units, where *S. enteritidis* infection was detected in the birds, were shown to excrete the organism for up to 18 weeks (Davies and Wray 1995a). Excretion was intermittent and reactivation of infection occurred during periods of stress. Salmonella contamination in the environment may be amplified by mice defaecating into feed troughs and on egg collection belts and may be spread throughout the house by automated feeding, egg conveyors and manure removal equipment.

Salmonella infection has been detected in many species of wild birds and, although poultry houses are often protected against wild bird entry, wild bird droppings may contaminate clean equipment left outside buildings (Davies and Wray 1994a).

Flies have frequently been shown to be contaminated with salmonella. Edel and others (1973) found that 1.5% of 202 flytraps examined were contaminated with salmonella. Blowfly larvae (*Lucilia serricata*) can carry salmonella and studies have shown that maggots are a potent vehicle of salmonella infection for chickens (Davies

and Wray 1994b). It has been suggested that mealworm beetles (*Alphitobius diaperinus*) may also be important in persistence and transmission of salmonella infections on poultry units (Baggesen and others 1992, Brown and others 1992).

Environmental contamination

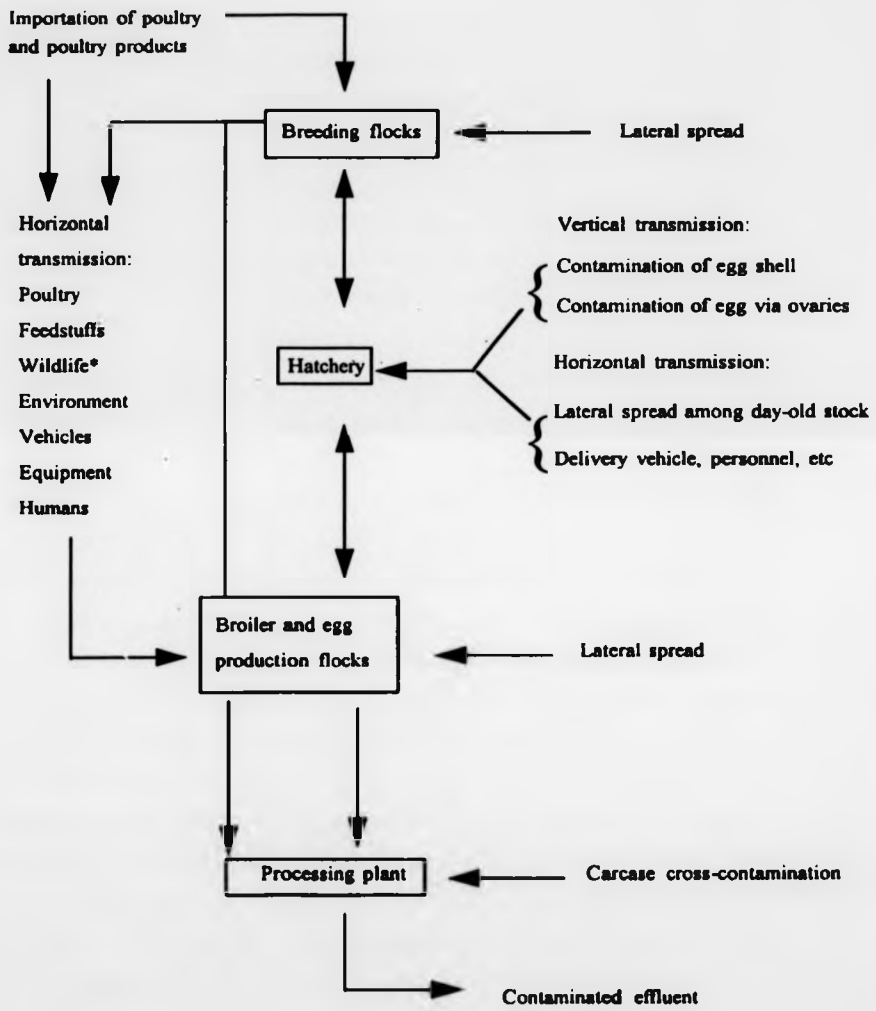
Persistent environmental contamination of houses is an important factor in the maintenance of *S. enteritidis*, and other salmonellae, in poultry flocks (Kradel and Miller 1991, Baggesen and others 1992, Angen and others 1996). A high standard of disinfection is necessary to avoid infection of poultry placed in previously infected houses as it has been shown experimentally that an infective dose of salmonella for chickens can be less than five cells (Milner and Shaffer 1952) or 100 cells for adult birds following conjunctival inoculation (Humphrey and others 1992). Studies have identified many potential problems during disinfection of poultry units naturally contaminated with *S. enteritidis* (Davies and Wray 1995b). Variations in the efficacy of commonly used disinfectants were apparent within a disinfectant group. It was possible for salmonella contamination to be amplified during preliminary washing and high numbers of salmonella were likely to persist in the absence of effective terminal disinfection. The efficacy of the disinfection regimen was not directly dependent on the standard of physical cleaning, if this was carried out to an adequate standard, because elimination of salmonella could be achieved even in the presence of substantial quantities of residual organic matter. Regimens involving formaldehyde, either as part of a terminal compound or as a fogging agent, were found to be the most effective. A number of analytical studies have associated salmonella infection with poor hygiene standards at poultry sites (Opitz 1992, Henzler and Opitz 1992, Henken and others 1992, Fris and van den bos 1995). Humans can also act as mechanical carriers of salmonella on contaminated clothing, footwear and hands.

Although many sources of salmonella infection for poultry are established, and have been discussed, the relative importance of these in the field is not known. It is likely that the major routes of infection may vary by serotype and flock type and their relative risk may have changed over time. Figure 2.2 shows a proposed cycle of salmonella infection in poultry established from evidence published in the literature.

Prevention and control

There are three major points at which poultry-associated human cases of *S. enteritidis* infection can be controlled. These are the prevention of infection in the live bird, slaughterhouse interventions to control contamination of carcasses and education of the public as to the necessity of adequate cooking of chicken meat and eggs and the prevention of cross-contamination of other foods in the kitchen. There are currently no acceptable slaughterhouse interventions that will ensure salmonella free meat and consumer food hygiene education has had only limited success. Therefore, control of the epidemic of *S. enteritidis* is centred on the eradication of infection in poultry. It is necessary to eliminate infection both from breeding and production flocks and experience from control schemes in various countries has shown that a "top down" approach, by first controlling infection in breeding flocks to prevent vertical transmission of infection to progeny, was most successful.

Government policy in Great Britain has been directed at regular monitoring of flocks and hatcheries. Infected birds, which may be a source of infection for other birds through vertical transmission or a direct source of human infection through the production of contaminated eggs, have been culled. Measures have also been taken to improve hygiene in hatcheries and on farms to limit the horizontal spread of *S. enteritidis* infection. A voluntary Code of Practice has been issued for the prevention and control of salmonella in breeding flocks and hatcheries (MAFF 1993) emphasising the importance of good hygiene practice and disease security. Feedstuffs and finished feed are subject to regular statutory monitoring to reduce the risk of introduction of infection through this source. Heat and chemical treatment of feed to control salmonella contamination are known to be effective, providing adequate temperatures and treatment times or adequate levels of chemicals are used (Jones and Richardson 1996). A recent Government report strongly recommended the effective heat treatment of all poultry feeds (HMSO 1996).



* Wildlife includes vermin, wild birds and insects

Figure 2.2. Cycle of salmonella infection in poultry

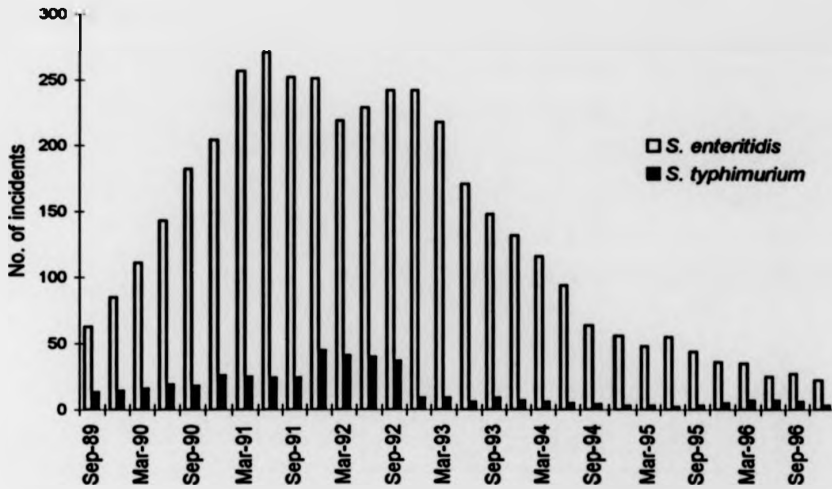


Figure 2.3. Salmonella incident reports in British broiler breeding flocks in 12 month periods (1989 - 1996)

Source: MAFF

There is now evidence that the measures to eradicate infection from the British poultry industry have had some success. Primary breeding flocks are free of infection and there is a declining trend in reports from parent breeding flocks (figures 2.3 and 2.4). However, eradication is still likely to be some time distant. Therefore, attention has been directed at interventions to protect birds from infection. The most feasible are competitive exclusion, antibiotic treatment and vaccination. Competitive exclusion refers to the colonisation control in the live bird by the establishment of protective populations of intestinal bacteria (Nurmi and Rantala 1973). Despite success under experimental conditions, it has shown mixed results in the field in its ability to protect against salmonella infection (Goren and others 1988, Mead 1991, Mulder and Bolder 1991). In general, protection is superior with undefined cultures that contain a broad range of bacteria (Stavric and others 1991). There is also a risk of spreading pathogens to recipient birds. The use of antibiotic treatment is controversial due to the risk of selection of resistant strains of bacteria (particularly

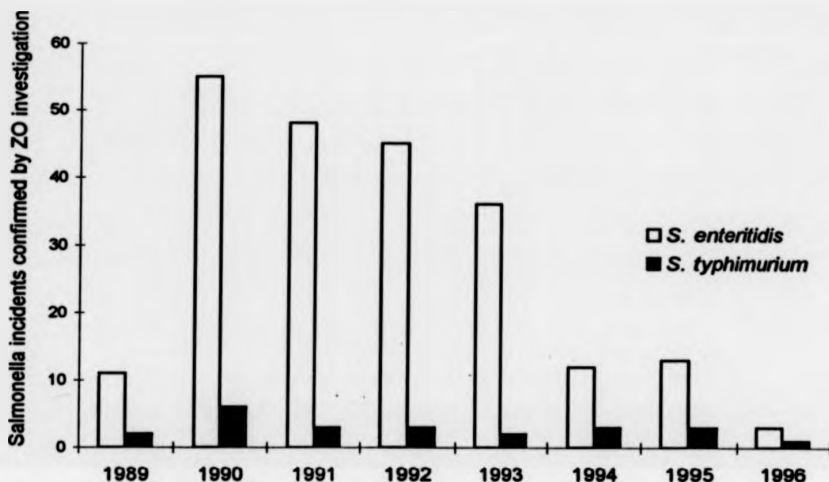


Figure 2.4. Confirmed salmonella incidents in broiler breeding flocks in Great Britain (1989 - 1996)

(NB: ZO = Zoonoses Order 1989)

Source: MAFF

if quinolone drugs are used). Recent trials in British breeding flocks infected with *S. enteritidis* have shown that a combination of antibiotic treatment and competitive exclusion reduced the prevalence of infection but was not successful in totally eliminating the organism (Reynolds and others 1997). Control by vaccination is still in the development stages although an inactivated vaccine has been available in recent years in Great Britain and has been used in breeding flocks. One disadvantage to vaccination is the interference with the results of serological monitoring of flocks for infection. All three methods of intervention are likely to be most successful when used as part of a comprehensive salmonella control programme.

Research needs

The literature contains few reports of epidemiological studies using field data and taking into account several potential risk factors for flock infection with salmonella at the same time. Some studies have analyzed infection with all salmonella serotypes

as the outcome of interest (Renwick and others 1992, Angen and others 1996). This complicates interpretation of the findings as there is evidence that different serotypes have different pathogenesis (Bisgaard and Hansen 1994) and the relative importance of risk factors may vary by serotype. The literature contains only one report of an analytical study specific to *S. enteritidis* infection of poultry. This was a retrospective case control study of *S. enteritidis* infections on Dutch broiler breeding farms which found no particular key factors associated with infection but concluded that a combination of factors influenced the risk of infection. These were the flock size and chicken breed, hygiene factors (site visitors, feed silo cleansing and disinfection and farm yard disinfection) and the proximity to farms with livestock other than poultry or animal processing industries (Fris and van den Bos 1995). However, only two of the variables (flock size and breed of bird) were associated with infection at the conventional level of significance ($p < 0.05$). There have been no studies of this type in Great Britain.

There are a number of problems to be addressed when designing analytical studies of salmonella infection in poultry. There are many potential routes of infection for poultry flocks as salmonella has a wide host range and survives well in the environment. Therefore, studies should be of sufficient size to detect fairly small relative risks which may result from the multifactorial nature of infection. The necessity to examine large numbers of flocks in order to detect small relative risks is a particular problem given the intensive structure of the poultry industry which is supported by relatively small numbers of large sized poultry units. For example, there are only a total of 600 breeding flocks in Great Britain. The lack of clinical signs of *S. enteritidis* infection in poultry causes problems in case ascertainment as does the frequency of low prevalence infection within flocks. Intensive sampling is therefore required to detect infection with a reasonable degree of certainty and a dependable system of case finding is necessary. As previously mentioned, the relative importance of the different sources of infection may vary by serotype and possibly also by phage type and the epidemiological picture will depend on the type of poultry enterprise investigated. A further potential complication is the temporal nature of the importance of the various sources of infection for poultry resulting from



the effects of on-going control policies (such as the relative importance of vertical transmission during the epidemic period).

Conclusion

Despite considerable efforts to control salmonella infection in poultry the problem is persistent. Analytical studies are required to assess the relative importance of risk factors for infection in order to best direct control efforts. Production flocks remain frequently infected with *S. enteritidis*, as indicated by retail surveys of chicken meat and table eggs, as do the parent breeding flocks. An essential point of control would appear to be the control of infection in parent breeding flocks to eliminate the potential for vertical transmission of infection to progeny. Thus, a case control study of *S. enteritidis* infection in parent breeding flocks, utilising the statutory monitoring scheme as a method of case ascertainment, would seem to be an efficient study design to address the problem. The aim would be to direct control efforts against the most important identified risk factors for infection in order to control infection in breeding flocks and thus prevent transmission of infection to progeny.

CHAPTER 3

A case control study of *Salmonella enteritidis* infection in British poultry breeding flocks.

* Submitted for publication in amended form as:
Evans S.J, Sayers A.R. Avian Pathology.

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Summary

A case control study of *Salmonella enteritidis* infection in poultry breeding flocks was undertaken to identify risk factors for infection. Information about management practices and disease security in flocks in which *S. enteritidis* infection was confirmed, as the result of statutory monitoring for salmonella in Great Britain between January 1992 and March 1994, was compared with similar information obtained from control flocks. The latter were flocks which had been monitored for salmonella throughout the study period with no evidence of infection. Where results of phage typing were available, 95% of case flocks were found to be infected with *S. enteritidis* phage type 4.

Data were analyzed from 277 parent breeding flocks (50% of the population), 90% of flocks were broiler breeders and the remainder were layer breeders. The age of the birds was positively associated with the risk of *S. enteritidis*. The risk of *S. enteritidis* infection increased with a concurrent rise in number of *S. enteritidis* incidents reported from the egg-destination hatchery. The risk of infection was also increased if *S. enteritidis* infection had been detected in a previous flock housed on the poultry site although this association may have been subject to bias. The presence of other domestic animals on the site increased the risk of infection. The use of heat treated poultry feed was associated with a two-fold reduction in risk of *S. enteritidis* infection. Standards of hygiene at the site did not appear to be associated with risk of infection. There was no evidence of vertical transmission of infection from grandparent flocks.

The most important measures identified by this study that are likely to reduce the risk of *S. enteritidis* PT4 infection in parent breeding flocks are thorough cleansing and disinfection of sites following a salmonella incident, strict hygiene measures between the site and the hatchery, effective isolation of the poultry unit from other domestic species and the use of heat treated poultry feed.

Introduction

The epidemic rise in number of cases of human salmonellosis in Great Britain reported since the mid 1980's was due to the emergence of *Salmonella enteritidis* phage type 4 as the most common strain involved in salmonella food poisoning (figure 1.1). Epidemiological investigations identified fresh shell eggs and poultry meat as major sources of infection (Coyle and other 1988, Cowden and others 1989, Roberts and Sockett 1994).

The human epidemic was mirrored in the poultry population (figure 2.1) and all sectors of the industry were affected, probably in part due to the ability of *S. enteritidis* to be transmitted vertically to progeny (O'Brien 1988, Lister 1988). In 1989 the British Government established a compulsory programme for the regular testing of poultry breeding flocks and commercial egg laying flocks for salmonella. When *S. enteritidis* or *S. typhimurium* was isolated from routine monitoring samples an investigation of a statistical sample of birds from the flock of origin was carried out and the flock was slaughtered if the presence of infection was confirmed. Controls on egg laying flocks ended in 1993 but a slaughter policy remains for breeding flocks. A "top down" approach concentrating on control of infection in breeding stock, thereby ensuring the delivery of salmonella free chicks to production flocks, forms the current strategy. There is evidence that primary British breeding flocks are now free of infection (HMSO 1996) but, although there has been a recent decline in incidence, infection is still reported from broiler and layer parent breeding flocks (MAFF 1996a). These flocks may be acquiring infection from horizontal routes such as the feed or the environment but the importance of these, and other potential sources of *S. enteritidis*, have not been established. There is only one published study of *S. enteritidis* infection in breeding flocks which has used field data and taken into account several potential risk factors. This was a retrospective case control study of broiler breeding flocks in The Netherlands which found no particular key factors associated with *S. enteritidis* but concluded that a combination of factors influenced the risk of infection. These were the flock size and poultry breed, hygiene factors and proximity to farms with livestock or animal processing industries (Fris and van den Bos 1995). The present study aimed to investigate the risk factors for

S. enteritidis infection in British parent breeding flocks at the height of the epidemic using data generated by the control policy. There have been no previous studies of this kind of the epidemiology of *S. enteritidis* in British poultry flocks.

Materials and methods

Study population

The study population was all registered fowl parent breeding flocks with at least 25 birds in England, Wales and Scotland (approximately 600 in total). These were flocks which were subject to statutory monitoring for salmonella as defined by the Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989 (Appendix A.2).

Statutory monitoring for salmonella

In 1989, a new Zoonoses Order (Statutory Instrument 1989 No. 285) replaced and broadened the scope of the previous order which was first enacted in 1975. The main provisions of the Order are the requirement to report the results of tests which identify the presence of salmonella, the provision of a culture to MAFF, the taking of live birds and other samples for diagnostic purposes, imposition of movement restrictions and isolation requirements, as well as a requirement for the cleaning and disinfection of premises and vehicles. The Order also makes provision for the compulsory slaughter and compensation of salmonella infected poultry flocks.

To combat *S. enteritidis* infection in breeding flocks, the Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order (Statutory Instrument 1989 No. 1963) was enacted in 1989. This Order required that poultry breeding flocks were subject to regular compulsory monitoring for salmonella throughout their productive life, both by direct monitoring of the flock at the poultry site and through offspring monitoring at the egg-destination hatcheries. This Order was revoked in 1993 with the implementation of the Poultry Breeding Flocks and Hatcheries Order (Statutory Instrument 1993 No. 1898), which brought salmonella control measures in poultry into line with the European Union Directive 92/117/EEC (Anon 1993a). Full details regarding the requirements of these Orders can be found in Appendices A.1 - A.3.

An isolation of *S. enteritidis* as the result of monitoring triggered a follow-up investigation in the flock of origin by veterinary officers from MAFF (England and Wales) and the Scottish Office Agriculture and Fisheries Department (Scotland). The protocol for this normally involved an initial serological screening of 59 birds from each house by an *S. enteritidis* lipopolysaccharide ELISA (Nicholas and Cullen 1991). No further action was taken if all birds were serologically negative but if any birds were ELISA positive at screening the flock was revisited to collect 59 whole birds for post mortem culture of the ovary, liver and intestine to identify whether the birds were currently infected. Microbiological examination of birds in flocks with serological evidence of antibodies to *S. enteritidis* was sometimes not possible as the flock had been depopulated. Salmonella isolates were serotyped and phage typed as appropriate.

Study design

Case control methodology was employed in the study to identify risk factors associated with flock infection with *S. enteritidis* in this population. Figure 3.1 shows a schematic diagram of the origin of case and control flocks for this study. Flocks were allocated to the highest ranked outcome consistent with the results of monitoring and follow-up investigation. Some breeding sites were investigated on more than one occasion during the study and the flock with the highest ranked outcome and most recent investigation was chosen for analysis.

Case definition

Cases were broiler breeding or layer breeding flocks detected as infected with *S. enteritidis* by the monitoring procedure described above between 1 January 1992 and 31 March 1994.

Control definition

The control group were flocks which had been monitored for salmonella throughout the study period with no evidence of infection. These included both flocks which were investigated as the result of an isolation of *S. enteritidis* during routine monitoring but were found to be serologically negative for salmonella and a random

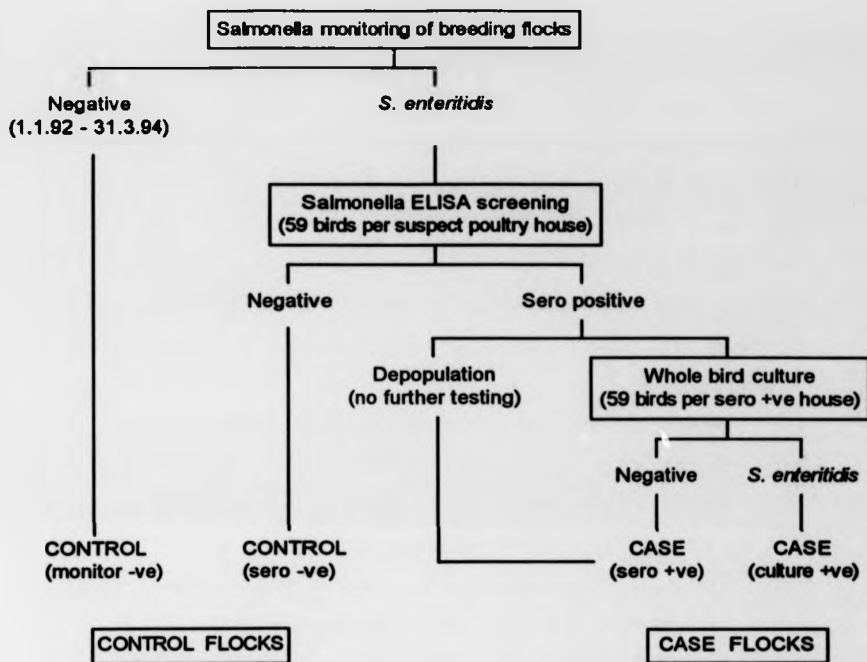


Figure 3.1. Schematic diagram of origin of case and control flocks

sample of flocks where all salmonella monitoring samples were negative during the study period. Most flock investigations were triggered as the result of monitoring at the egg destination hatchery and it was considered likely that hatchery cross-contamination was largely responsible for the false positive monitoring results of the former group of flocks.

In order to investigate whether these resulting case and control groupings were appropriate for analysis, salmonella status was also defined as an ordinal dependent variable containing four flock outcome categories ranked from low to high as follows:

1 Salmonella monitoring negative;

Result of salmonella monitoring follow-up investigations: -

2 Salmonella sero-negative;

3 *S. enteritidis* sero-positive;

4 *S. enteritidis* culture positive.

Data collection

Exposure data were collected by a report form which was completed at all flock follow-up investigations for salmonella (Appendix A.4). It included information relating to:-

- 1 Source of chicks and date of arrival at site, number of birds and current age;
- 2 Disease security policy for staff at site - protective clothing, hygiene facilities;
- 3 Disease security policy related to site visitors including delivery vehicles;
- 4 Poultry buildings - state of repair, hygiene barriers, clean out procedures;
- 5 Presence of other species on /near site - domestic animals, rodents, wild birds;
- 6 Feed supplier and feed delivery procedures;
- 7 Salmonella preventive measures - additional voluntary monitoring, examination of chick suppliers test programme, feed treatments (heat, acid, probiotic, antibiotic);
- 8 Results of serology / bacteriology.

A different form was used at flock investigations prior to 1 January 1993. Therefore, for the purposes of the study, the new form was completed retrospectively for flock investigations carried out during 1992 (Appendices A.5 - A.6). Exposure information for the sample of flocks with negative monitoring for salmonella during the study period was collected towards the end of the study period, usually by visiting the flock (Appendices A.7 - A.9). The disease status of these flocks was not assessed as it was assumed that the probability of infection at the time of the visit was relatively small due to the regular monitoring. To identify any bias that may exist from management practices changing over time, an additional section of the questionnaire completed by these negative-monitored flocks recorded any changes in disease security policy or management at the site since the start of the study period (Appendix A.9).

Historical salmonella monitoring information was also used to define a number of variables for each flock which were hypothesised to be associated with the risk of *S. enteritidis* infection. These included:-

- 1 From the results of monitoring at the egg-destination hatchery:-
 - the number of *S. enteritidis* incidents reported during 1993;
 - the incubator capacity.
- 2 From the past results of monitoring at the poultry breeding site:-
 - whether the preceding flock was subject to a follow-up investigation;
 - whether any previous flock housed on the site was investigated for salmonella since the commencement of compulsory monitoring in 1989;
 - whether any previous, except the preceding, flock was investigated (this variable was created to limit bias that may have been introduced by using a control group of flocks which were selected on the basis that all monitoring was negative during the study period).

Statistical analysis

Model 1

Multiple logistic regression was used to identify the exposure variables which were significantly associated with *S. enteritidis* infection using the case control outcome defined and data from all flocks. Each exposure variable was screened for inclusion in the initial model by examining its association with *S. enteritidis* infection in a univariate analysis. Those associated with infection at $p < 0.20$ and all *a priori* confounders (age of birds, number of birds in the flock and time between the detection of *S. enteritidis* by the routine monitoring process and the commencement of the follow-up flock investigation) were incorporated into a multiple logistic regression in the statistical package EGRET (Anon 1993b) to identify which variables had an effect on outcome either independently or through interaction with another variable. In order to avoid problems of model instability due to collinearity, groups of highly correlated variables were represented by single summary variables. Variables with more than 20% missing values were also excluded at this stage. A backward elimination procedure was used to remove non-significant variables ($p > 0.05$) until all those remaining in the model were significant. Interactions among

these variables and with variables not in the model were tested and included if significant at $p \leq 0.01$. Finally, the model was extended to test in turn all excluded variables, including those with missing values. Any variable whose addition to the model resulted in a significant ($p \leq 0.05$) likelihood ratio test statistic (LRS) or altered the estimated coefficients of one or more of the main risk variables by at least 50% was included in the final model.

The fraction of disease in the population that would not have occurred if the risk factor of interest was absent is referred to as the population attributable risk per cent (PAR). The PAR was estimated for each of the risk factors using the method of Bruzzi and others (1985) and the logistic regression model without interactions. A summary PAR was calculated for all main risk factors acting together.

Model 2

A second model was formed which was restricted to flocks which were subject to follow-up investigations for salmonella during the study period. The purpose of this model was to assess whether similar results were obtained when monitor-negative flocks were excluded from the analysis and to identify any biased associations which may have arisen from the inclusion of these flocks in the control group.

Models 3 and 4

Two further models were constructed using the 4-level ordinal outcome described above. The first of these assigned scores 1-4 to the ordinal responses and modelled them using the estimated generalized least squares (EGLS) method described by Lipsitz (1992). The second model was a proportional odds model (McCullagh 1980) which was fitted using the "ordinallogistic" procedure in the statistical package GENSTAT 5 (Payne and others 1987).

Results

Table 3.1 shows the number of individual parent breeding sites available for analysis and their salmonella status. Ninety per cent of flocks in the study were broiler breeding flocks, the remainder being layer breeding flocks.

Table 3.1. Salmonella status of flocks in study

Flock outcome	n	%		n	%
Salmonella monitor negative	97	35.0	} control	183	66.1
Salmonella follow-up investigation:					
Salmonella sero-negative	86	31.1			
<i>S. enteritidis</i> sero-positive	36	13.0	} case	94	33.9
<i>S. enteritidis</i> culture positive	58	20.9			

The average flock size in the study was 16,000 birds (standard deviation = 11,200) and these were housed in an average of 3-4 poultry houses each containing an average of 4,500 birds (standard deviation = 2,800). Ninety five per cent of sites housed birds of a single age and operated on an all in all out basis. At least 75% of flocks were reared at specialist sites and moved to the breeding site at 18 weeks of age.

***S. enteritidis* culture positive flocks**

The 58 sites with *S. enteritidis* culture positive birds contained 133 poultry houses with evidence of infection in the birds. The estimated prevalence of infected birds in the houses ranged from 1.8-45.9% (mean = 16.4%, standard deviation = 16%). It was estimated that less than 10% of birds were infected with *S. enteritidis* in over 40% of houses (figure 3.2). Phage typing of *S. enteritidis* isolates was performed in 55 of the 58 culture positive investigations. *S. enteritidis* PT4 was isolated from 91% of flocks but in 14 flocks (25%) other phage types were also found (1, 4A, 5A, 6A, 7, 7A) and in 5 flocks (9%) only other phage types were detected (1, 4A, 5A, 24). Occasionally other serotypes, mainly *S. typhimurium*, were also detected (6%).

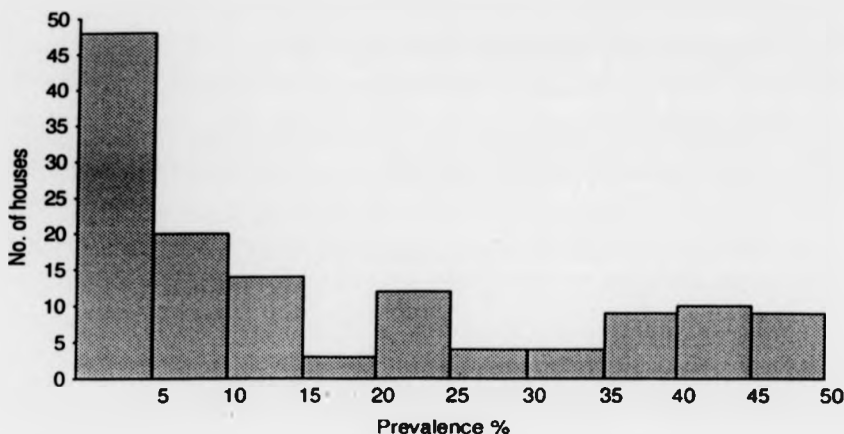


Figure 3.2. Prevalence of *S. enteritidis* culture positive birds in culture positive flocks

S. enteritidis sero-positive flocks

There was serological evidence of *S. enteritidis* infection of birds in 79 poultry houses contained on 36 sites. The seroprevalence of ELISA positive birds in these houses ranged from 1.7-96.6% (mean = 37.3%, s.d = 27.2%) (figure 3.3).

The age of the birds at the onset of *S. enteritidis* infection was unknown but was inferred from the age of the birds at the time of the initial salmonella monitoring isolation, which averaged just over 40 weeks for case flocks with a standard deviation of 14 weeks (figure 3.4), and the fact that the majority of follow-up investigations resulted from trace back from the monitoring of hatching chicks. Therefore, the parent birds were likely to have been infected at least 4 weeks earlier (Corkish and others 1994). Thus, the age-specific incidence of *S. enteritidis* infection in breeding flocks was estimated to be greatest when the birds were 24-48 weeks of age.

The response rate for the provision of retrospective information for 1992 follow-up

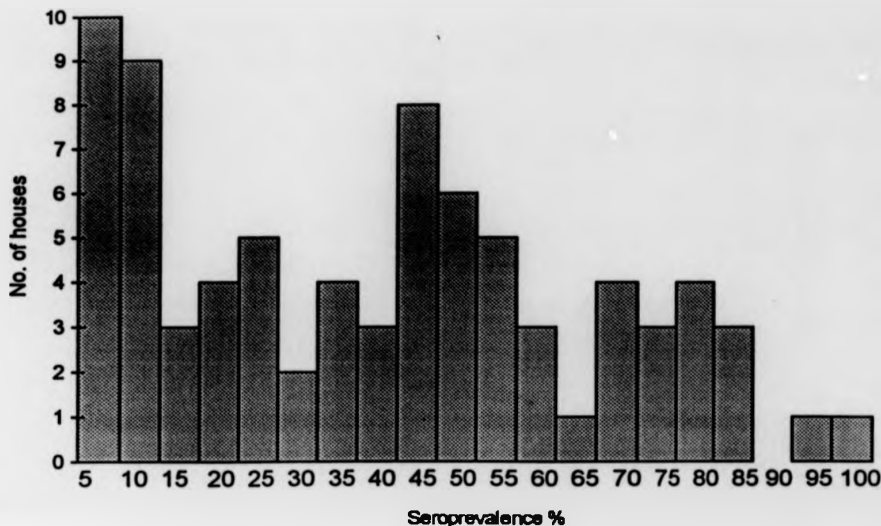


Figure 3.3. Seroprevalence of *S. enteritidis* ELISA positive birds in seropositive flocks.

investigations was 94%, and 95% of owners of randomly selected negative-monitored flocks, who were approached, agreed to provide comparison information.

Univariate analysis

Model 1

Table 3.2 gives the results of the univariate analysis using data from all flocks and a binary case control outcome and lists the 31 variables found to be associated with *S. enteritidis* infection at $p < 0.20$. Of these, 15 variables were associated with infection at $p \leq 0.05$, including all *a priori* confounders, and the remainder at $0.05 < p < 0.20$. The effects of those variables associated at $p \leq 0.05$ are described below and the effects of the others are shown in table 3.2.

The region in which the farm was situated was associated with the risk of infection

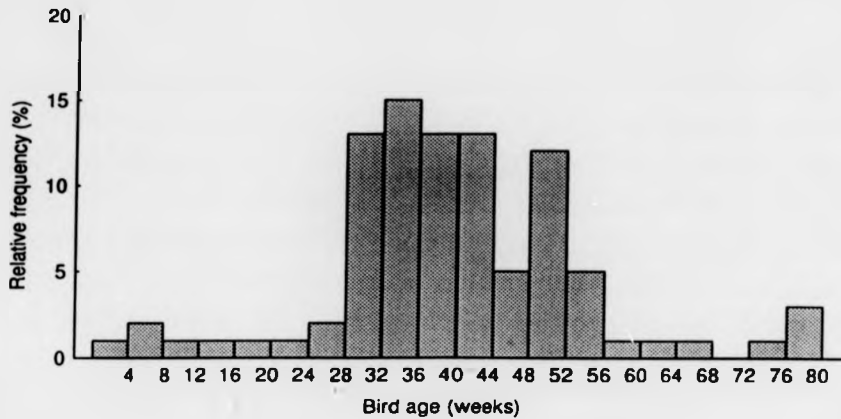


Figure 3.4. Age of birds at first detection of *S. enteritidis* infection

and this was attributed to a slightly greater proportion of cases in south west England and Wales and few cases in Scotland. There was a seasonal association with infection but this may have been biased by the fact that information on negative-monitored control flocks was collected during the winter and spring of 1993/4. The risk of infection was greater in medium to large sized flocks compared with small or very large flocks and there was a positive association between the age of the birds and the risk of infection. There was also a positive association with the risk of infection and the time that had elapsed between the initial monitoring isolate of *S. enteritidis* and the flock follow-up investigation. All variables relating to a history of salmonella at the poultry site were positively associated with the risk of infection as was the variable relating to the results of salmonella monitoring at the egg-destination hatchery. Heat treatment of poultry feed was strongly protective against infection and the presence of other domestic species on the site increased the risk of flock infection. Sites which logged all visitors in a visitors book were at lower risk of infection and this variable was correlated with a number of other disease security variables. More than 95% of sites power washed houses between flocks but those which did not were

at greater risk of infection. There was an unexpected univariate association between the presence of a secure perimeter fence around the site and an increased risk of infection although less than 10% of sites were fenced.

The following variables were not associated with the risk of *S. enteritidis* infection in the univariate analysis ($p > 0.20$): proximity to other poultry sites (65% of sites were less than 1 kilometre from other poultry), whether chicks were reared on the breeding site or elsewhere, site visitor hygiene precautions, vehicle entry hygiene precautions, age and state of repair of the poultry houses, the presence of cattle on the site, most poultry building clean out procedures, additional voluntary salmonella monitoring (69% of flocks carried out additional tests), the inclusion of animal protein in the diet (42% of flocks were fed diets containing animal protein) and acid or antibiotic treatment of the feed (58% and 11% of feeds were reported to be treated by these methods respectively). There was no association with antibiotic or probiotic treatment of the breeding flock in the previous 28 days although 93% of flocks had not received either. There were too many individual egg-destination hatcheries and feed mills listed to examine their association with infection.

Model 2

The following variables were not univariately associated with infection ($p > 0.20$) when the analysis was restricted to the 180 flocks which were subject to salmonella follow-up investigations during the study period: region, season, bird age, the presence of cats or dogs on the site, the poultry houses protected against wild bird entry, visitors logged in a visitors book, power washing poultry houses or steam cleaning fixtures between flocks, the use of a chlorinated water supply, a shower available for staff use, the renewal frequency of the boot dip disinfectant solution and the use of home mixed feed or feed treated by heat or the addition of probiotics. Evidence of rodent activity on the site as scored by the number of signs reported (live, dead, droppings, damage, burrows) was associated with the risk of *S. enteritidis* infection at $p = 0.130$ in this restricted analysis. However, the association was in the opposite direction to that which was expected (evidence of rodent activity was associated with an apparent reduction in the risk of infection).

Table 3.2. Results of univariate analysis using data from all flocks (all variables associated with *S. enteritidis* infection at $p < 0.20$ and *a priori* confounders)

Exposure	All flocks (% exposed)	Case	Control	OR	p-value
Poultry site location					
Northern England	23 (8.3)	6	17	1.000	0.002
Mid & West England	44 (15.9)	12	32	1.063	
Eastern England	104 (37.5)	40	64	1.771	
South East England	29 (10.5)	9	20	1.275	
South West England	31 (11.2)	16	15	3.022	
Wales	17 (6.1)	9	8	3.188	
Scotland	29 (10.5)	2	27	0.210	
Season of farm visit/questionnaire completion					
Winter	94 (33.9)	30	64	1.000	0.025
Spring	96 (34.7)	24	72	0.711	
Summer	48 (17.3)	21	27	1.659	
Autumn	39 (14.1)	19	20	2.027	
Flock size (no. of birds)					
≤10,000	83 (30.0)	22	61	1.000	0.044
10,001-20,000	127 (45.8)	49	78	1.742	
20,001-30,000	44 (15.9)	19	25	2.107	
>30,000	23 (8.3)	4	19	0.584	
Average age of birds on site (weeks)					
≤20	25 (9.0)	5	20	1.000	0.009
21-30	25 (9.0)	2	23	0.348	
31-40	74 (26.7)	27	47	2.298	
41-50	81 (29.2)	35	46	3.043	
51-60	55 (19.9)	18	37	1.946	
>60	17 (6.1)	7	10	2.800	

Table 3.2. (continued)

Exposure	All flocks (% exposed)	Case	Control	OR	p-value
Time between initial <i>S. enteritidis</i> isolation & flock follow-up investigation (weeks)					
≤2	41 (22.9)	18	23	1.000	0.033
3-4	80 (44.7)	36	44	1.045	
5-6	39 (21.8)	25	14	2.282	
≥7	19 (10.6)	14	5	3.578	
(χ^2 test for trend=7.11 p=0.008)					
Salmonella investigation in preceding flock					
No	234 (84.5)	65	169	1.000	<0.001
Yes, sero-negative	16 (5.8)	10	6	4.330	
Yes, sero/bact positive	27 (9.7)	19	8	6.180	
(χ^2 test for trend =24.56 p<0.001)					
Salmonella investigation since 1989					
No	173 (62.5)	41	132	1.000	<0.001
Yes, sero-negative	45 (16.2)	24	21	3.680	
Yes, sero/bact positive	59 (21.3)	29	30	3.110	
(χ^2 test for trend =17.10 p<0.001)					
Salmonella investigation since 1989 excluding preceding flock					
No	199 (71.8)	59	140	1.000	0.048
Yes, sero-negative	37 (13.3)	18	19	2.250	
Yes, sero/bact positive	41 (14.8)	17	24	1.680	
(χ^2 test for trend =4.00 p=0.045)					
Incubator capacity of hatchery (million eggs)					
≤0.5	69 (29.0)	19	50	1.000	0.117
>0.5-0.75	53 (22.3)	24	29	2.593	
>0.75-1.0	32 (13.4)	12	20	1.843	
>1.0	96 (40.3)	35	61	1.798	

Table 3.2. (continued)

Exposure	All flocks (% exposed)	Case	Control	OR	p-value
<i>S. enteritidis</i> incident reports at hatchery (1993)					
None	57 (22.3)	11	46	1.000	<0.001
1-10	58 (22.7)	17	41	1.734	
11-20	61 (23.8)	19	42	1.892	
21-30	34 (13.3)	22	12	7.667	
>30	46 (18.0)	20	26	3.217	
(χ^2 test for trend =13.47 p<0.001)					
Hatchery supplying chicks (grouped when appropriate e.g. other countries, more than one source)					
A	43 (15.5)	14	29	1.000	0.051
B	43 (15.5)	17	26	1.354	
C	34 (12.3)	7	27	0.537	
D	57 (20.6)	15	42	0.740	
E	70 (25.3)	33	37	1.847	
F	30 (10.8)	8	22	0.753	
Chick suppliers salmonella test programme examined					
No	45 (17.9)	10	35	1.000	0.073
Yes	207 (82.1)	74	133	1.947	
Pelleted feed used					
No	191 (70.5)	70	121	1.000	0.059
Yes	80 (29.5)	20	60	0.576	
Probiotic used					
No	229 (84.5)	72	157	1.000	0.155
Yes	42 (15.5)	18	24	1.635	
Heat treated feed used					
No	187 (69.0)	75	112	1.000	<0.001
Yes	84 (31.0)	15	69	0.325	

Table 3.2. (continued)

Exposure	All flocks (% exposed)	Case	Control	OR	p-value
Feed delivery in dedicated vehicles					
No	12 (4.5)	2	10	1.000	0.179
Yes	253 (95.5)	87	166	2.620	
Home mixed feed used					
No	261 (96.7)	89	172	1.000	0.116
Yes	9 (3.3)	1	8	0.242	
Other domestic species on site					
No	161 (60.1)	43	118	1.000	0.006
Yes	107 (39.9)	46	61	2.069	
Cats/dogs on site					
No	182 (67.9)	53	129	1.000	0.040
Yes	86 (32.1)	36	50	1.752	
Sheep on site					
No	243 (90.3)	84	159	1.000	0.097
Yes	26 (9.7)	5	21	0.448	
Livestock other than cattle/sheep on site					
No	255 (94.8)	81	174	1.000	0.058
Yes	14 (5.2)	8	6	2.860	
Staff contact with other poultry					
No	239 (90.9)	84	155	1.000	0.053
Yes	24 (9.1)	4	20	0.369	
Mice/rat droppings seen					
No	186 (69.9)	67	119	1.000	0.116
Yes	80 (30.1)	21	59	0.632	

Table 3.2. (continued)

Exposure	All flocks (% exposed)	Case	Control	OR	p-value
Poultry houses protected against wildbird entry					
No	21 (8.0)	11	10	1.000	0.073
Yes	243 (92.0)	79	164	0.438	
Secure perimeter fence around site					
No	246 (91.4)	77	169	1.000	0.018
Yes	23 (8.6)	13	10	2.853	
Visitors always logged in a book					
No	129 (48.3)	53	76	1.000	0.014
Yes	138 (51.7)	37	101	0.525	
Shower available for staff					
No	250 (92.9)	86	164	1.000	0.078
Yes	19 (7.1)	3	16	0.358	
Chlorinated water supply					
No	57 (21.8)	24	33	1.000	0.128
Yes	205 (78.2)	64	141	0.624	
Powerwash poultry houses between flocks					
No	11 (4.1)	7	4	1.000	0.036
Yes	256 (95.9)	82	174	0.269	
Steam clean fixtures between flocks					
No	179 (67.3)	55	124	1.000	0.178
Yes	87 (32.7)	34	53	1.446	
Renewal frequency of disinfectant in boot dips					
>Weekly	80 (30.7)	31	49	1.000	0.188
≤Weekly	181 (69.3)	55	126	0.690	

Multivariable analysis

Model 1

The results of examining the influence of multiple variables on the risk of *S. enteritidis* infection together in a single model of a case control outcome are given in tables 3.3 and 3.4. A final model was formed of seven variables found to be independently significantly associated with the risk of *S. enteritidis* infection ($p \leq 0.05$) using the full data set (table 3.3). The effects of the two main identified interactions between model variables ($p \leq 0.01$) are shown separately (table 3.4).

The age of the birds was positively associated with the risk of infection and was included in the model as a confounder. Flock size was not significantly independently associated with infection and so was excluded from the final model. It was not appropriate to model the time that had elapsed between the initial monitoring isolate and the flock follow-up investigation due to the inclusion of negative-monitored control flocks in the analysis for which this variable had no relevance.

The risk of *S. enteritidis* infection was associated with the number of *S. enteritidis* incidents reported at the egg-destination hatchery. The increase in risk occurred when the hatchery reported more than 20 incidents annually. Hatchery size (as indicated by incubator capacity) was not significantly independently associated with infection but was positively associated with hatchery monitoring ($p < 0.0001$). When interactions were excluded from the model, a previous follow-up investigation for salmonella at the poultry site (irrespective of outcome) increased the risk and the use of heat treated poultry feed reduced the risk of *S. enteritidis* infection. When the interaction between these two variables was modelled it was shown that there was a significant increased risk of infection if there had been a previous confirmed incident of *S. enteritidis* at the poultry site and the feed was not heat treated compared with sites which were monitored for salmonella with no evidence of infection and which used heat treated feed. Sites with a history of a previous follow-up investigation for salmonella but there was no evidence of infection (birds were sero-negative) were at increased risk of infection (odds ratio = 12.28, 95% confidence limits: 1.613-93.43) compared with sites which were monitor-negative, if heat treated feed was used by

both site types. Heat treated feed significantly protected against infection (OR=0.015, 95% C.L: 0.007-0.301) on sites where there had been a previous reported incident of *S. enteritidis*.

The presence of other domestic animals on the poultry site was generally associated with an increased risk of infection except in the situation when cattle were the only other species (which had no effect on risk) and when sheep and cats or dogs were present together on the site (the risk of infection was reduced compared with sites which did not have sheep, OR=0.039, 95% C.L: 0.004-0.432). The presence of sheep was correlated with antibiotic treatment of the flock ($p=0.004$). Only 9% of poultry sites without sheep on the site had recently treated the flock with antibiotics compared with 31% of sites with sheep.

The variation explained by the regression as measured by the square of the Pearson correlation between the binary outcome and the predictor was 31.7%. Population attributable risks were estimated for the main risk factors in the model, without fitting the interactions. The main risk factors for *S. enteritidis* infection of British parent breeding flocks identified in this study, listed in descending order of importance, were the absence of sheep on the site (PAR=0.682), the use of feed which had not been heat treated (PAR=0.532), a previous investigation for salmonella at the site (PAR=0.360), more than 20 incidents of *S. enteritidis* reported annually at the egg-destination hatchery (PAR=0.328), the presence of cats or dogs on the site (PAR=0.212) and the presence of livestock other than cattle or sheep on the site (PAR=0.079). However, interpretation is complicated by the interactions between variables. The summary PAR was close to one as none of the flocks in the study reported an absence of all of the risk factors.

Sixty five per cent of negative-monitored control flocks reported recent management or disease security changes at the site. Twenty per cent of sites had changed the type of feed used for the poultry and 13% had changed feed supplier. General hygiene standards had been improved on 16% of sites and 10% of sites reported that poultry houses had been refurbished. Ten per cent of sites had changed chick supplier and

4% of sites had changed egg-destination hatchery. However, after the exclusion from the analysis of negative-monitored control flocks which changed to using heat treated feed during the study period, heat treatment of feed was still significantly protective through interaction with a history of salmonella follow-up investigation at the site.

Model 2

Similar results were found when the analysis was restricted to investigated flocks. However, a history of salmonella at the site was not significant in this multivariable model although it was associated univariately with infection at $p=0.053$. Heat treatment of poultry feed was significantly protective through interaction with the renewal frequency of the disinfectant solution in the boot dips. Heat treatment of the feed was significantly protective against infection when the boot dip solution was changed more frequently than once weekly (OR=0.031, 95% C.L: 0.003-0.370) and changing the boot dip solution less frequently appeared to be protective when heat treated feed was not used (OR=0.400, 95% C.L: 0.167-0.958). Evidence of rodent activity, as scored by the number of signs reported, was independently associated with the risk of infection in this analysis. Sites which reported more than three of the possible five signs of rodent activity were less likely to be infected compared with sites with no evidence of rodent activity (OR=0.154, 95% C.L: 0.048-0.495). The time between the initial monitoring salmonella isolate and the flock follow-up investigation was not significantly independently associated with infection and therefore did not confound the results.

Models 3 and 4

When the data were modelled with an ordinal outcome, both approaches used gave broadly similar results with the variables in the models being significant in at least one of the models using a binary outcome.

Table 3.3 Multivariable analysis - adjusted odds ratios for *S. enteritidis* infection in breeding flocks (model 1, without interactions)

Exposure	All flocks (% exposed)	Case (%) n=85	Control n=163	Adjusted OR	95% CL
Salmonella investigation since 1989					
None	155 (62.5)	36 (23.2)	119	1.000	
-ve only	41 (16.5)	23 (56.1)	18	3.071	(1.303-7.240)
≥ +ve	52 (21)	26 (50)	26	2.408	(1.118-5.185)
					(LRS on 2 df=8.85, p=0.012)
<i>S. enteritidis</i> incident reports at hatchery (1993)					
None	54 (21.8)	10 (18.5)	44	1.000	
1-10	57 (23)	16 (28.1)	41	0.900	(0.316-2.567)
11-20	61 (24.6)	19 (31.1)	42	0.967	(0.347-2.693)
21-30	31 (12.5)	20 (64.5)	11	5.355	(1.564-18.33)
>30	45 (18.1)	20 (44.4)	25	2.210	(0.780-6.268)
					(LRS on 4 df=15.01, p=0.005)
Cats/dogs on site					
No	171 (69)	49 (28.7)	122	1.000	
Yes	77 (31)	36 (46.8)	41	2.003	(1.009-3.976)
					(LRS on 1 df=3.96, p=0.047)
Sheep on site					
No	224 (90.3)	80 (35.7)	144	1.000	
Yes	24 (9.7)	5 (20.8)	19	0.273	(0.084-0.890)
					(LRS on 1 df=5.27, p=0.022)
Livestock other than cattle/sheep on site					
No	237 (95.6)	77 (32.5)	160	1.000	
Yes	11 (4.4)	8 (72.7)	3	6.329	(1.153-34.75)
					(LRS on 1 df=5.26, p=0.022)

Table 3.3. (continued)

Exposure	All flocks (% exposed)	Case (%) n=85	Control n=163	Adjusted OR	95% CL
Heat treatment of poultry feed					
No	176 (71)	72 (40.9)	104	1.000	
Yes	72 (29)	13 (18.1)	59	0.368	(0.165-0.824)
(LRS on 1 df=6.37, p=0.012)					
Age of birds (weeks)					
0-20	14 (5.6)	1 (7.1)	13	1.000	
21-30	20 (8.1)	2 (10)	18	1.423	(0.102-19.95)
31-40	67 (27)	24 (35.8)	43	7.081	(0.759-66.10)
41-50	78 (31.5)	34 (43.6)	44	9.823	(1.084-88.97)
51-60	53 (21.4)	17 (32.1)	36	3.800	(0.396-36.41)
>60	16 (6.5)	7 (43.8)	9	13.070	(1.163-146.8)
(LRS on 5 df=15.05, p=0.010)					

Table 3.4. Multivariable analysis - effects of interactions between variables (model 1)

3.4a. Heat treatment of poultry feed and the history of salmonella infection on the poultry site since 1989 (LRS on 2df=6.403, p=0.011)

Exposure	Case	Control	Adjusted OR ¹	95% CL
Negative monitoring and non-heat treated poultry feed	30	79	1.000	
Negative previous salmonella investigation and non-heat treated poultry feed	19	16	2.464	0.934-6.504
Previous <i>S. enteritidis</i> incident at site and non-heat treated feed	26	17	3.639	1.471-9.001
Negative monitoring and heat treated feed	8	51	0.524	0.190-1.445
Negative previous salmonella investigation and heat treated feed	5	5	6.434	0.962-43.03
Previous <i>S. enteritidis</i> incident at site and heat treated feed	2	13	0.053	0.003-1.027

¹ Odds ratio adjusted for the number of *S. enteritidis* incidents reported at the egg destination hatchery in 1993, the presence of cats, dogs, sheep or other livestock (other than cattle) on the site and the age of the birds.

Table 3.4. (continued)

3.4b. Presence of cats or dogs and sheep on the poultry site

(LRS on 1 df=11.847, p=0.003)

Exposure	Case	Control	Adjusted OR ¹	95% CL
No cats/dogs or sheep on site	49	123	1.000	
Cats/dogs on site but no sheep	35	35	3.074	1.443-6.548
Sheep on site but no cats/dogs	4	6	1.121	0.229-5.476
Cats/dogs and sheep on site	1	15	0.119	0.011-1.280

¹ Odds ratio adjusted for previous investigation for salmonella since 1989, *S. enteritidis* incident reports at the hatchery (1993), the presence of livestock (other than cattle/sheep), the use of heat treated poultry feed and the age of the birds.

Discussion

The main risk factors for *S. enteritidis* infection of poultry parent breeding flocks in Great Britain identified by the case control study were those associated with horizontal routes of salmonella transmission at the breeding sites. These included contaminated feed and infection due to persistently contaminated buildings, the presence of other domestic animals on the sites and possible spread of infection from visits to the site by hatchery equipment and personnel. There was no evidence of vertical transmission of infection from grandparent flocks. Infections during the rearing period were rare and the risk of infection increased with age.

The reasons for the recent pandemic of *S. enteritidis* infection in man and poultry are not clear, neither is why different phage types have become dominant in different areas of the world (Rodrigue and others 1990). However, it is apparent that the British poultry industry has been heavily infected with *S. enteritidis* PT4 since the mid 1980's and only recently has there been evidence that the epidemic is in decline as the result of the stringent control measures introduced by MAFF. Experience from this epidemic has led scientists to identify three major areas of potential salmonella contamination of poultry stock. These are: infection of the breeding stock and resultant vertical transmission of infection to progeny, feed contamination and environmental contamination (Barrow 1993). The present study used data from breeding flocks collected at the time of the peak of the *S. enteritidis* epidemic to identify which of the major routes of infection were most important at this time. The results were specific to *S. enteritidis* PT4 which was isolated from over 90% of cases. Nearly 50% of the British population of registered breeding flocks were included in the study. The results can be generalised to the parent population without fear of bias as all identified cases of infection were included in the analysis, the negative-monitored control flocks were randomly selected and the participation rate was excellent. However, the results may not be applicable to smaller "back yard" flocks of less than 25 breeding birds, which were not included in this study.

The case definition used for the study included both *S. enteritidis* sero-positive and culture positive flocks. The control group consisted of both salmonella investigated

flocks which were found to be non-infected and flocks with negative monitoring results through-out the study period. The data were modelled using a binary outcome but, as it was not initially clear whether it was appropriate to combine these groupings, further models were constructed with each of the four flock outcomes separated. These analyses showed that, as expected, sero-positive and culture positive flocks did not differ markedly from each other but that there were some differences between salmonella investigated sero-negative flocks and negative-monitored flocks. The results of the analysis using the case control outcome and data from all 277 flocks (model 1) have been reported as this was the simplest model to interpret and had the greatest explanatory value.

The identification of *S. enteritidis* infected birds is difficult because infection usually induces a chronic carrier state where birds intermittently excrete the organism (Williams 1972). The mean prevalence of ELISA positivity within flocks in the study was considerably greater than the prevalence of infection estimated by culture. This finding was confirmed by other workers when both serum samples and whole birds were taken from the same flock (Nicholas and Cullen 1991, Corkish and others 1994). This is because the presence of antibody can indicate intermittent excretion or past infection and does not always indicate active infection with *S. enteritidis*. It is known that antibody titres can persist for at least one year after detectable excretion of *S. enteritidis* has ceased (Nicholas and Cullen 1991). However, assuming management procedures did not change during the life of the flock, the timing of infection should not bias the risk factor analysis. Some cross-reactivity between *S. enteritidis* and *S. typhimurium* in the *S. enteritidis* ELISA can also occur (Nicholas and Cullen 1991) but it is unlikely that this will have biased the results as *S. typhimurium* was known to be far less common than *S. enteritidis* (figure 2.1) and all flocks were investigated as the result of *S. enteritidis* being cultured from a monitoring sample. Some infected flocks may have been wrongly classified as non-infected as the salmonella screening methods were less than 100% sensitive but this probability will have been minimised by the regular compulsory monitoring throughout the life of the flock and the fact that most suspect flocks were subject to both serological and bacteriological investigation. The effect of this type of

misclassification is to reduce the strength of true associations rather than produce false associations.

Standards of flock management are likely to have improved over time as the industry became more aware of the salmonella problem and this was shown by the study as 65% of control flocks visited at the end of the study period reported improvements in disease security since the start of the study. Concern that this temporal effect may have biased the association between heat treatment of feed and the risk of infection was investigated by excluding control flocks from the analysis which had changed to heat treatment during the study period. Heat treatment of feed remained protective in their absence indicating that the association was not biased by time.

Few flocks were detected as infected during the rearing period so it seemed unlikely that vertical transmission of infection from grandparent flocks was a major route of infection at this time. This was confirmed by the lack of association between the hatchery of origin of the chicks and salmonella infection in the flock. Other studies have also concluded that vertical transmission of *S. enteritidis* to parent breeders does not appear to be important, at least in The Netherlands in recent years (Fris and van den Bos 1995). Therefore, one must assume that infection is introduced to these flocks via the feed or from the environment. Less than 10% of the cases were detected when flocks were less than 28 weeks of age and most infections (77%) were detected during the first half of the laying period when birds were between 28 and 52 weeks of age. This age distribution at detection of infection was similar to that seen in a Dutch survey of *S. enteritidis* in commercial laying flocks (van de Giessen and others 1994). The authors of the survey concluded that vertical transmission was not a major source of infection to Dutch laying flocks at the time of the study as infections during the rearing period were rare. They suggested that the farm environment was the most important factor in infection (transmission via improperly cleansed and disinfected poultry houses or infected vermin) as they considered that the probability of infection from this source would decrease with time producing a cumulative infection curve similar to that which was observed, whereas they predicted that infections from the external environment (such as feed) would produce a linear

increase in infection. The present study found that both the farm environment and feed were potential routes of transmission of *S. enteritidis* to British breeding flocks.

The farm environment appeared to be important in the epidemiology of *S. enteritidis* in this study as three separate factors which may allow horizontal transmission of the bacterium were found to be significantly associated with the risk of infection. The most important of these appeared to be a history of salmonella at the poultry site and this may indicate that the organism was surviving in the environment or in animal reservoirs around the site to infect a subsequent flock of birds. The tendency for re-infection on the same farm is widely recognised as is the importance of adequate cleansing and disinfection and rodent control (Kradel and Miller 1991, Baggesen and others 1992, Opitz 1992, Fris and van den Bos 1995, Davies and Wray 1995a, Davies and Wray 1995b). A retrospective study of Danish broiler flocks also associated salmonella infection with the salmonella status of the preceding flock (Angen and others 1996). Salmonella are resistant to desiccation and have been shown to survive many months in empty farm buildings (Bale and others 1993). However, the present study was surprisingly unable to identify a specific effect of hygiene. This may reflect the difficulty in measurement of hygiene standards, as individual variation in operator ability may be an important factor but is difficult to assess by questionnaire, or the standardised nature of hygiene practices in this country. A report of a pilot project examining the epidemiology of *S. enteritidis* in laying flocks was also unable to associate infection with specific management practices (Mason 1994). It should be remembered that good hygiene practice alone will not prevent infection in the presence of feed contamination or vertical transmission. The association with a history of salmonella at the site was not significant when the analysis was restricted to salmonella investigated flocks (model 2) indicating that control selection bias cannot be ruled out as an alternative explanation for this finding. However, this association may have been non-significant as the result of a reduction in the power of the analysis due to the smaller data set. This restricted analysis produced two rather unexpected differences between salmonella infected and sero-negative flocks. These were an apparent increased risk associated with more frequent replenishment of the boot dip solution outside poultry

houses and a protective effect associated with the reported presence of rodents on the site. These may be chance findings due to the smaller number of flocks in the analysis but, alternatively, may have resulted from the nature of the study which examined both exposure and disease status at the same point in time. Therefore, sites which were aware of a potential salmonella problem may have been more likely than controls to increase disease security around poultry houses (by increasing the frequency with which they replenished the boot dip solution) and may also have had reason to identify and control a potential source of infection (rodents). Also, other studies have shown that rodent populations were often poorly assessed by farm managers (Mason 1994, Davies and Wray 1995b). The study showed an association between the incidence of *S. enteritidis* infection at the egg-destination hatchery and the risk of infection in the breeding flock. Cross-contamination within the hatchery has been reported as a risk factor for infection in broiler flocks (Bailey and others 1994, Davies and Wray 1994a, Angen and others 1996) but may also constitute a risk to breeding flocks through the use of inadequately disinfected trays and trolleys that are returned to breeder units (Davies and Wray 1994a). Therefore, the frequent visits to the sites by hatchery personnel, vehicles and equipment may have been responsible for the introduction of infection to the flock. However, this association may have been subject to some degree of control selection bias. The presence of other domestic animals, including cats and dogs, on the site increased the risk of infection which indicated that these animals may be carriers of infection. However, the majority of breeding sites were not involved in other farm enterprises and therefore not exposed to this risk factor. In addition, the small number of farms which kept poultry, sheep and cats or dogs were less likely to be infected with salmonella. There was no obvious explanation from the data for this finding although there was a greater tendency for antibiotic usage in these farms which may have prevented infection.

Poultry feed was associated indirectly with infection in this study through the apparent protective effect of heat treatment of feed. Heat treatment, at a sufficiently high temperature, is known to be an effective method of removing salmonella from feed (Cox and others 1986, Voeten and van de Leest 1989, Ekperigin and others 1991, Veldman and others 1995) and although MAFF monitoring indicates only a low level

of *S. enteritidis* contamination of poultry feed in this country (MAFF 1996a) existing legislation does little to prevent feed, potentially contaminated with salmonella, from entering the food chain. In addition, one cannot rule out the possibility that small numbers of *S. enteritidis* bacteria are contaminating batches of feed and escaping detection, particularly in the light of the heterogenous nature of contamination (Veldman and others 1995) and the ability of the organisms to multiply from non-detectable numbers during improper feed storage (Davies 1992). After all, the most sensitive identifiers of salmonella in poultry feed are likely to be the birds consuming the food. Investigation of the feasibility of heat treatment of all poultry feed was recommended by the Lamming Report on animal feedstuffs (HMSO 1992) and subsequently a MAFF Code of Practice for the prevention and control of salmonella in breeding flocks recommended that heat or other effective anti-salmonella treatment should be used in the manufacture of feed intended for breeding flocks (MAFF 1993) but in the current study less than a third of flocks received heat treated feed. Historically, heat treated feed has not been popular for breeding flocks due to the increased energy density of the feed and therefore the tendency for the birds to become overweight. However, it is possible to reduce the energy density of the ration formulation to compensate for heat treatment and this should make the method more popular. There is also a cost implication which must be borne. Sweden has for a number of years recommended that poultry feed is heat treated as part of its comprehensive salmonella control policy (Wierup and others 1995) and all breeder feed has been heat treated in Northern Ireland since early epidemiological investigations revealed that feed was a possible source of salmonella for these flocks (McIlroy and others 1989). Both Sweden and Northern Ireland have successfully controlled salmonella in their poultry industries. Other recent studies have also implicated feed as a possible source of salmonella for poultry flocks although this risk may be associated with serotypes other than *S. enteritidis* (Henken and others 1992, Jacobs-Reitsma and others 1994, Angen and others 1996). The present study found no significant protective effect of other reported methods used to decontaminate poultry feed.

The final model (model 1) explained less than a third of the differences between cases

and controls so other factors, not examined by the questionnaire or which have only small individual influences on the risk of infection, may be important. The multifactorial nature of the epidemiology of *S. enteritidis* infection in poultry is clear. No one preventive measure will be effective in controlling salmonella infection within the poultry industry but this study has highlighted areas with the greatest potential impact on the incidence of infection in the population.

There was no evidence that chicks were infected on arrival at the site, infection of flocks occurred through horizontal routes of transmission at the breeder sites. To summarise, the most important measures identified by the study that are likely to reduce the incidence of *S. enteritidis* PT4 infection in poultry breeding flocks in Great Britain are thorough cleansing and disinfection of sites following a salmonella incident, strict hygiene measures between the site and the egg-destination hatchery, effective isolation of the poultry unit from other animal species and the use of heat treated poultry feed.

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**2 EPIDEMIOLOGICAL STUDIES OF
THERMOPHILIC CAMPYLOBACTER
INFECTION IN BRITISH BROILER
FLOCKS**

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CHAPTER 4

Thermophilic campylobacter infection of poultry

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Introduction

Thermophilic campylobacters are found in the intestinal tracts of a wide variety of animals and birds often without causing disease. They are not major veterinary pathogens and their main significance lies in the ability of infected animals to serve as reservoirs of infection for human disease. The reasons for the differences in pathogenicity between animals and man are not known. The three main species of thermophilic campylobacters are *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari*.

All three species can be isolated from poultry but the main species is *C. jejuni*. Current evidence points to these bacteria existing in the intestinal tract of poultry as non-pathogenic commensals. However, in the mid 1960's a new disease syndrome in laying flocks, called Avian Vibrionic Hepatitis, was recognised and subsequently attributed to infection with thermophilic "vibrio-like" organisms. This condition has since disappeared and there is now doubt as to whether campylobacters were the cause as experimental studies have repeatedly failed to induce hepatopathy in chicks inoculated with *C. jejuni* (Doyle 1991).

Methods of detection

Campylobacters are fragile, fastidious, slow-growing organisms. The principal niche for colonisation in the bird is the caeca and caecal contents are the diagnostic sample of choice. However, cloacal swabs or fresh faecal samples are also suitable for the detection of infection as infected birds shed large numbers of campylobacters in their faeces. Samples should be sent to the laboratory without delay and stored at 4°C. A transport medium is beneficial if samples can not be processed straight away or the specimen is likely to contain only a few organisms. Direct examination is not used routinely due to low sensitivity. However, polymerase chain reaction-based restriction fragment length polymorphism (PCR/RFLP) analysis of flagellar genes may prove useful for rapid detection and typing purposes in the future. Latex agglutination kits are also available but should only be used for confirmation purposes. A solid selective media, containing antibiotics to inhibit unwanted organisms, is used routinely for isolation of the organisms. A pre-enrichment broth

can be used to increase sensitivity. Plates are incubated micro-aerophilically, at 42-43°C, for 48-72 hours. Colonies are non-haemolytic, round, smooth and greyish-white in colour. Despite their inability to ferment or oxidise carbohydrates, there are a number of recommended tests for the identification of *Campylobacter species* including oxidase, catalase, nitrate and nitrite reduction, urease, hydrogen sulphide production, hippurate hydrolysis, indoxyl acetate hydrolysis and testing for sensitivity to nalidixic acid and cephalothin.

There are a number of different typing schemes which can be used to identify different strains within species but they are restricted to reference laboratories and there is no correlation between different schemes. Currently, the most widely used schemes are biotyping and serotyping. Biotyping, based on further biochemical tests and resistance patterns, is simple to perform but has limited ability to discriminate between strains so it is best used in combination with other typing methods. Serotyping, based on heat stable or heat labile antigens, is limited by the lack of availability of the large number of antisera required but has been the method of choice for research purposes. Molecular techniques such as PCR/RFLP are currently in development and, if quantifiable and relatively simple and inexpensive to perform, they may gain wide popularity in the future.

Epidemiology of infection

Large numbers of campylobacters can be present in the avian intestinal tract without any apparent gross pathology (Stern and others 1988). There is currently no recognised clinical syndrome in poultry attributed to infection with these bacteria. The purpose of controlling infection in poultry is to reduce the potential for food-borne transmission of the bacteria to humans.

There is no evidence that campylobacteriosis in man is attributable to the consumption of table eggs. Therefore, this review concentrates on the epidemiology and control of infection in broiler flocks as consumption or handling of table chicken has been identified as a major source of campylobacter infection for man.

Prevalence

Prevalence of infection in broiler breeding flocks has been found to be as high as 70-80% (Shanker and others 1986, Jacobs-Reitsma 1995) but campylobacters are rarely isolated from hatcheries or newly hatched chicks (Engvall and others 1986, Jacobs-Reitsma and others 1995a). The prevalence reported in broiler production flocks varies, possibly owing to variations in age, isolation technique or season. The few surveys that have been conducted have shown that the prevalence also varies considerably between countries. Surveys in The Netherlands and Norway reported that 82% of 187 and 18% of 176 investigated broiler flocks respectively were infected with campylobacters at slaughter (Jacobs-Reitsma and others 1994, Kapperud and others 1993). A recent study of a limited number of broiler flocks in Sweden found 27% of flocks and 39% of production cycles were infected with campylobacter in a one year study period but 16 of the 18 sites (89%) housed infected birds in at least one production cycle during the year (Berndtson and others 1996a). Production systems in Great Britain more closely resemble systems in The Netherlands than Scandinavia, where poultry management is less intensive. The prevalence of infection in Great Britain has not been established although a limited study of one integrated broiler company in England found 76% of flocks were infected (Humphrey and others 1993). These surveys report *C. jejuni* as the most frequently isolated species from poultry but occasionally *C. coli* and *C. lari* are found. Flocks may be infected with more than one species of campylobacter and within species multiple strains are commonly found in an infected flock (Jacobs-Reitsma 1995, Jacobs-Reitsma and others 1995b).

Descriptive studies have shown that broiler flocks usually become infected without showing clinical signs when the chicks are 3-5 weeks old but infection has been observed as early as 7 days of age. Once campylobacter is isolated from a flock, all birds become rapidly colonised and remain so up to the time of slaughter, usually at 6-7 weeks of age (Genigeorgis and others 1986, Jacobs-Reitsma and others 1995a). The organism is isolated in large numbers from the majority of birds sampled. Experimentally, chicks have been shown to cease shedding the bacteria three months after challenge. However, the short lifespan of the broiler chick precludes this

natural self-limitation of infection. The reason for the delay in colonisation is not known. Experimental studies suggest that broiler chicks are equally susceptible to campylobacter infection throughout their lifespan (Stern and others 1988, Shanker and others 1990, Kazwala and others 1992) so it appears unlikely that young chicks in commercial flocks are inherently resistant to the organisms. Coprophagy may partially explain the rapid spread of infection within flocks and there is also evidence that transient palatine carriage may result in spread via communal drinking water systems (Montrose and others 1985, Shanker and others 1990).

Sources of infection

Vertical transmission

Vertical transmission of infection from parent breeding flocks seems unlikely to occur as eggs and newly hatched chicks from infected breeding flocks have been found to be free of campylobacter (Engvall and others 1986, Shane and others 1986, Shanker and others 1986, Jacobs-Reitsma and others 1995a). Experimental studies have also shown that *C. jejuni* does not easily penetrate the egg (Doyle 1984, Clark and Bueschkens 1985, Neill and others 1985, Shanker and others 1988). Typing studies have failed to show an association between strains carried by parent flocks and their offspring (Jacobs-Reitsma 1995). In contrast, a recently reported study of *C. jejuni* infection on a single broiler farm found an association between the hatchery supplier and the isolation rate of *C. jejuni* in the broilers studied and the authors concluded that low level vertical transmission was the most likely explanation (Pearson and others 1996). However, other potential confounding factors were not considered and, on balance, the bulk of the evidence to date does not support a route of campylobacter infection from parent to offspring via the egg.

Feed contamination

As anticipated, feed samples taken from broiler houses have not been found to contain campylobacters due to the low moisture content of poultry feed and the organisms sensitivity to drying (Annan-Prah and Janc 1988, Pearson and others 1993, Jacobs-Reitsma and others 1995a). Nevertheless, it has been shown experimentally that contaminated feed can introduce infection to young chicks (Al-Obaidi 1988).

Water

Drinking water may act as a vehicle of infection for growing broiler chicks (Engvall and others 1986, Kapperud and others 1993, Pearson and others 1993). Campylobacters survive well in cold water and human water-borne outbreaks have been widely reported (Mentzing 1981, Rogol and others 1983, Sacks and others 1986). Chlorination of the water supply has been shown to reduce the prevalence of *C. jejuni* in flocks supplied with water from a borehole (Pearson and others 1987) but, in Great Britain, the use of private non-chlorinated water supplies are relatively rare. However, even if the drinking water is chlorinated it should be ensured that the water header tanks and drinking equipment are kept clean as chlorine is rapidly inactivated by organic matter.

Domestic animals, wildlife and insects

C. jejuni is commonly carried by domestic and free-living animals found on farms including cattle, pigs, dogs, rodents and wild birds (Annan-Prah and Janc 1988, Kapperud and others 1993). These species have been shown to carry similar campylobacter serotypes as poultry. Unless stringent control measures are adopted, most poultry sites harbour a large rodent population. Rodent droppings may be particularly important sources of campylobacter infection for flocks especially if there is evidence of rodent access to poultry houses or food stores. Insects, including *Alphitobius species*, have been shown to be carriers of *C. jejuni* (Rosef and Kapperud 1983). It has been shown that houseflies can transmit *C. jejuni* to chicks in the laboratory but it is not known how important this process is in the field (Shane and others 1985).

Environmental contamination

Campylobacters can survive for extended periods in environmental niches in poultry units and poor hygiene standards on farms may allow infection to be introduced to flocks (Hoop and Ehrsam 1987) or to persist in successive production cycles. Infection may be spread by movement of personnel between broiler houses or farms. In support of this view, campylobacters have been recovered from the boots of farm workers and surface water near poultry houses (Annan-Prah and Janc 1988, Kazwala

and others 1990). Thorough cleansing and disinfection of broiler houses after depopulation is very important to prevent subsequent flocks becoming infected.

Broiler houses are usually depopulated over a number of days and it has been suggested that the risk of infection to remaining birds in the flock may be increased by the presence of processing plant personnel or equipment when birds are collected for slaughter (Jacobs-Reitsma and others 1994, Berndtson and others 1996b).

Prevention and control

Thermophilic campylobacters are commonly found in birds at slaughter and the caeca and intestines of infected birds have been shown to contain very large numbers of bacteria. The organism appears to survive the processing operation and cross-contamination during procedures such as scalding, plucking, evisceration and immersion chilling may even allow the prevalence of carcass contamination to exceed that of infection in the live bird (Simmons and Gibbs 1979, Oosterom and others 1983, Wempe and others 1983, Rogol and others 1985). Retail surveys have shown that typically more than 50% of chicken carcasses are contaminated (Stern and others 1984, Hood and others 1988, Flynn and others 1994). Super chlorination of the washing water, organic acid sprays, hot rinses and forced air chilling may reduce carcass contamination levels in the processing plant but are unlikely to achieve elimination (Mead and others 1995). The organism is also very sensitive to irradiation but this is not an option at present due to concerns over safety. Campylobacters are fragile organisms and are susceptible to drying (except when refrigerated), oxygen, direct sunlight and most disinfectants but they survive well in foods under refrigeration and can also survive in lower numbers on frozen foods. Unlike salmonellas, campylobacters will not replicate in foods stored below 30°C. Thermal inactivation occurs at 48°C. They will not survive pasteurisation or typical meat cooking procedures. However, consumer education and re-enforcement of hygienic practices at catering establishments have so far been inadequate to prevent human campylobacteriosis.

Thus the control of poultry associated campylobacter infections in man would appear

to depend on the control of infections in broiler flocks. Competitive exclusion has been investigated as a method to prevent salmonella and campylobacter colonisation of broiler chicks. However, the primary niche for *C. jejuni* colonisation is the mucin layer of the caeca and it is therefore necessary to develop a culture which contains organisms to compete for this niche. Recently, competitive exclusion flora derived from this mucosal layer have shown some protective ability against both salmonella and *C. jejuni* colonisation. Other potential approaches to intervention include chlorination of the drinking water and improved hygiene on the farms, selective breeding for resistance and immunisation (Stern 1992).

In the absence of cost-effective and acceptable carcass decontamination methods, the aim must be to produce birds free from infection at slaughter and so reduce the potential for human infection. The limited progress in the field of biological control methods has led workers to concentrate on elucidating the epidemiology of infection in the field. There is a particular need to identify risk factors for infection which may then allow the development of farm interventions, by which infection can be controlled, to achieve this aim.

Research needs

There are several practical problems to consider when interpreting the findings of the studies reviewed here. Campylobacters are relatively slow growing, fastidious bacteria which require specialised culture conditions, and the isolate must be typed for its source to be traced. There are many typing schemes, but they are restricted to reference laboratories owing to the expert technology required; they cannot be correlated with each other and they are not suitable for the identification of virulence which may, in any case, alter during the passage of the organism through a susceptible host (King and others 1991). Non-culturable injured campylobacters have been identified by the fluorescent antibody technique which can revert to culturable forms in favourable conditions (Rollins and Colwell 1986). This has led to the validity of previous studies being questioned. However, recent evidence suggests that these forms are unable to colonise chicks and are, therefore, not important (Medema and others 1992, Fearnley and others 1995, van de Giessen and others 1996a).

Studies have so far been limited to the exhaustive bacteriological sampling of small numbers of flocks, and these descriptive studies suffer from a number of limitations. The birds and the potential sources of infection are examined at the same point in time and it may therefore be difficult to separate cause and effect; investigators are often unable to detect sources of potential infection in uninfected flocks but when a flock is infected all the possible sources are found to be infected. Descriptive studies can be used to formulate hypotheses but analytical studies are necessary to test these hypotheses. Ideally, analytical studies should investigate flocks as a single farm unit rather than compartmentalising flocks into separate broiler houses. This is because of the uniform exposure of birds within the farm unit ("herd effect") which complicates data analyses. It is also important that appropriate numbers of flocks are examined to produce meaningful results. This requirement has been overlooked by some investigators who have been more concerned with sampling sufficient birds within each flock to detect low levels of infection; as a result the rapid within-flock transmission has, on occasion, been erroneously attributed to an inadequate sampling procedure. However, a random sample of only 29 birds is required to be 95 per cent certain of detecting infection in a flock of 20,000 birds if only 10 per cent of the birds are infected.

Conclusion

The transmission of *C. jejuni* infection to broiler flocks may be due to a multiplicity of factors. Different factors may be dominant at different times and the occurrence of different serotypes within flocks indicates the complexity of the epidemiology. Analytical studies are required to test the aetiological hypotheses that have been put forward. Initially a well designed cross-sectional survey which uses adequate numbers of flocks and classifies the exposures accurately may be the most appropriate to identify the risk factors for infection. The importance of identified risk factors can be established in a case control or cohort study. The objective would then be to avoid these risks during broiler production in order to produce campylobacter-free chickens for slaughter and thus reduce the potential for the spread of campylobacteriosis from poultry to man.

CHAPTER 5

The evaluation of sampling and typing techniques for epidemiological studies of thermophilic campylobacter infection of poultry.

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Evans S.J, Ayling R.D, Newell D.G. *Epidemiology and Infection*

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Summary

A study has been conducted to develop appropriate sampling methods for the detection of campylobacter infection in broiler chicken. Twenty broiler flocks were screened for campylobacter at 5-6 weeks of age. *Campylobacter jejuni* was detected in 10 flocks and it was estimated that 90-100% of the birds in 8 of these flocks were colonized. Collection of caecal droppings compared with cloacal swabs did not improve the sensitivity of screening. Seven campylobacter-negative flocks were screened again when the birds were slaughtered and over half were infected with *C. jejuni*. Examination of caeca compared with cloacal swabs did not affect the sensitivity of screening. Infection was related to the stage of depopulation of the broiler house; the first batch of birds slaughtered was less likely to be infected than subsequent batches. It was hypothesised that during the collection of birds for slaughter contaminated equipment or personnel may introduce campylobacter infection to remaining birds.

Introduction

Broiler chicken are frequently infected with thermophilic campylobacters, mainly *C. jejuni* (Prescott and Munroe 1982, Tauxe and others 1985, Hood and others 1988, Humphrey and others 1993), and the consumption or handling of chicken has been considered a major risk factor for human campylobacteriosis (Harris and others 1986, Deming and others 1987). Infection of poultry is not generally associated with clinical illness even though large numbers of campylobacters are excreted in the faeces. Descriptive studies have shown that broiler flocks usually become infected when the chicks are three to five weeks old (Smitherman and others 1984, Engvall and others 1986, Hoop and Ehram 1987) and infection spreads rapidly to most birds within the flock. Birds then remain carriers through to slaughter at six or seven weeks of age (Smitherman and others 1984, Annan-Prah and Janc 1988, Shanker and others 1990). The prevalence of campylobacter infection in broiler flocks in the United Kingdom has not been established and the epidemiology of infection in poultry requires further investigation (Evans 1992). In particular, the source or sources of infection remain unknown. The ultimate aim must be to produce birds free from infection at slaughter and so reduce the potential for human infection from this source. The identification of risk factors for campylobacter infection may allow the development of suitable farm interventions to achieve this aim. The present study was conducted to develop appropriate sampling procedures and laboratory protocols for the detection of campylobacter infection in broiler flocks.

Materials and methods

Data collection

Twenty broiler farms associated with two integrated broiler producing companies in England were involved in the study. Farms were visited by the author between January and April 1993 when one or more of the poultry houses on the site contained birds which were at least five weeks of age but before any of the birds from the current production cycle had been slaughtered. On each site, 60 cloacal swabs (in Amies transport media with charcoal) were collected from birds in one randomly selected broiler house, housing birds which were at least 35 days old. The house was visually divided into 12 equal compartments and 5 birds were sampled from each

section. For comparison purposes, pooled faecal samples consisting of 5-10 fresh caecal droppings were collected in sterile universal containers from each quarter of the house and a portion of each pooled faecal sample was placed into 10 mls of Exeter campylobacter-selective medium (de Boer and Humphrey 1991).

Flocks which were campylobacter culture-negative were sampled again at the processing plant when birds from the investigated house were slaughtered. From each flock, 30 cloacal swabs were obtained from birds immediately prior to slaughter and 30 caeca were collected from the same batch of birds after evisceration. Caeca were transported to the laboratory on dry ice and stored at -70°C until examination.

Microbiological methods

The faecal samples and 30 of the cloacal swabs collected at the farm visits were cultured and if all were negative the second batch of 30 cloacal swabs, which had been stored at 4°C for up to 10 days, were examined. All cloacal swabs obtained at slaughter were cultured and caeca were examined if all the swabs were campylobacter culture-negative.

For bacteriological culture, each swab was placed in 10 mls of Exeter medium and incubated at 42°C , micro-aerophilically for 48 hours. A small amount of each faeces sample was examined in a similar manner. Following the enrichment step, 50 μl of Exeter culture was plated onto Blood agar plates containing Skirrows antibiotics (Skirrow 1977) with actidione (100 $\mu\text{l}/\text{ml}$) and cefoperazone (30 $\mu\text{l}/\text{ml}$) (BASAC) and incubated as before. Caeca were thawed and opened aseptically. A sample of caecal contents was placed directly onto a BASAC plate and a second sample was plated out following enrichment in Exeter medium. Plates were incubated as before and any suspect campylobacter colonies subcultured onto fresh BASAC plates to obtain pure cultures. A maximum of 10 isolates per flock were speciated (*C. jejuni*, *C. coli* or *C. lari*) using recommended techniques (Goossens and Butzler 1992). Isolates were serotyped by the methods of Penner and Hennessey (1980) and typed by a molecular technique based on the restriction fragment length polymorphism of polymerase chain reaction products (PCR/RFLP) of the flagellin genes (Ayling and others 1996).

Table 5.1. *C. jejuni* prevalence in broiler flocks when birds were 5-6 weeks old and 95% confidence limits

No. houses (%)	No. positive cloacal swabs	No. negative cloacal swabs	<i>C. jejuni</i> prevalence % (95 % CL)
10 (50)	0	60	0 (0.0 - 6)
1 (5)	3	57	5 (0.5 - 14)
1 (5)	23	7	77 (58 - 91)
8 (40)	30	0	100 (90 - 100)

Results

Ten of the 20 flocks tested (50%) were found to be colonized with *C. jejuni* when birds were 5-6 weeks of age. No other species of campylobacter was isolated. Table 5.1 shows binomial confidence limits for the within-flock prevalence of *C. jejuni* infection. At this age, it was estimated that 90-100% of birds in 80% of infected flocks were excreting campylobacters.

None of the faecal samples yielded campylobacters in the 10 flocks in which all cloacal swabs were negative. The campylobacter isolation rate from pooled faecal samples in positive flocks was 92.9% when samples were placed directly into Exeter medium at the farm compared with an isolation rate of 82.1% when samples were not placed into Exeter medium until arrival at the laboratory (usually the following morning). This difference was statistically significant at the 10% level of significance ($z=1.71$, $p=0.087$).

The results of the bacteriological investigation of the flocks at 5-6 weeks of age were available in time to arrange slaughter sampling of 7 of the 10 campylobacter-negative flocks. All 30 cloacal swabs and caeca examined at slaughter were negative in two of these flocks but campylobacters were cultured from most birds sampled in four flocks. *C. jejuni* infection was associated with the stage of depopulation of the broiler house. Both flocks which were campylobacter-negative at slaughter were screened

Table 5.2. Comparison of *C. jejuni* Penner serotypes and PCR/RFLP profiles detected in broiler flocks sampled at farm visits and at slaughter

Farm no.	Farm Visit:		Slaughter Sampling:	
	Penner	PCR/RFLP	Penner	PCR/RFLP
1	-	-	1,11,nt	1, 5, 3
2	1	1	ns	ns
3	-	-	1,7,nt	2,3
4	-	-	1,4,7,10,nt	1,2,3,5,13
5	2	2	ns	ns
6	2	2	ns	ns
7	-	-	-	-
8	4	2	ns	ns
9	-	-	ns	ns
10	-	-	10	5
11	nt	1,6	ns	ns
12	21	4	ns	ns
13	10,nt	4	ns	ns
14	1,nt	5	ns	ns
15	-	-	ns	ns
16	11	5,3	ns	ns
17	-	-	1,13	1,2
18	-	-	ns	ns
19	4,13,nt	2	ns	ns
20	-	-	-	-

-, campylobacter negative; ns, not sampled; nt, not typable.

when the first batch of birds were taken from the house for slaughter and three of the four infected flocks were screened when the final batch of birds were slaughtered. The final flock sampled was screened twice during depopulation of the house. Birds sampled from the first batch killed at 43 days of age were negative but birds remaining in the broiler house after this partial depopulation or "thin" were found to be infected with *C. jejuni* at the time of their slaughter at 49 days of age.

All 135 speciated isolates were *C. jejuni*. The results of further subtyping of the isolates are shown in table 5.2. Eight Penner serotypes were identified and, in most cases, each broiler house appeared to have one dominant serotype present. The number of different Penner serotypes detected in individual flocks ranged from 1-2 at farm visits and 1-4 when birds were sampled at slaughter. Penner serotype P1 was the most frequently observed and was isolated from 40% of flocks. Only 85% of isolates were typable by this method but all isolates were typable using the PCR/RFLP method. Seven PCR/RFLP profiles were distinguished with profile 2, detected in 47% of flocks, and profiles 1 and 5, each detected in 33% of flocks, being most common. The number of different PCR/RFLP profile types in each flock ranged from 1-2 at farm visits and 1-5 when sampled at slaughter.

Discussion

The prevalence of campylobacter infection in British broiler flocks is unknown and large scale random surveys are required to establish the level of infection with precision. The optimal protocols for such surveys need to be established and this study aimed to determine efficient sampling procedures for such purposes.

The sensitivity of cloacal swabbing, as a method of detection of campylobacter infection, was investigated by also collecting caeca, which are the primary niche for colonization, from birds sampled at slaughter. The three flocks in which all 30 cloacal swabs obtained at slaughter were campylobacter negative were also negative by culture of the caecal contents. In addition, pooled caecal droppings were always negative in broiler houses in which all 60 cloacal swabs were campylobacter culture-negative. Therefore, the collection of caecal droppings or whole caeca, compared

with cloacal swabs, did not appear to improve the sensitivity of screening for campylobacter infection in broiler flocks. The *C. jejuni* isolation rate from pooled caecal droppings was greater if the droppings were placed directly into Exeter medium at the farm than if transported to the laboratory without the use of media. The loss of campylobacter viability from faecal samples, especially without the use of Exeter media for transport, may be caused by desiccation or overgrowth of competing bacteria. In this study, sampling caecal droppings provided no advantage over cloacal swabs for campylobacter detection. A previous publication evaluating sampling methods concluded that caecal droppings were the most sensitive samples for assessing campylobacter colonisation in broiler chicken (Stern and Robach 1995). However, it is more difficult and time consuming to collect these samples than cloacal swabs and the improvement in sensitivity was not apparent when screening flocks rather than individuals for infection. Thus, for survey purposes, cloacal swabs are considered adequate; providing appropriate storage and transportation of clinical material while maintaining bacterial viability.

The study protocol involved sampling 60 individual birds from each flock in order to ensure that low prevalence infections would be detected with a reasonable degree of certainty. The results indicated that most birds in an infected flock were excreting campylobacters and therefore there was only a small probability of misclassifying a truly infected flock even if very few of the birds were sampled. To improve efficiency in future studies it is considered that only 15-20 birds would need to be sampled from each flock to provide a reasonable sensitivity of detection of flock infection with campylobacter.

The sources and routes of campylobacter infection in poultry remain debatable. Such epidemiological studies would be aided by the use of methods to subtype campylobacter isolates. Suitable subtyping methods must be simple, reliable and cheap to perform. They should not only allow adequate discrimination between strains but be widely applicable to veterinary isolates. *C. jejuni* isolates generated by this study were used to compare the performance of a molecular typing scheme (PCR/RFLP) with the widely used Penner serotyping scheme and these results have

been reported in full elsewhere (Ayling and others 1996). However, it was of interest to note that, with both typing schemes, a greater number of subtypes were identified when flocks were sampled at slaughter than when sampled at the poultry farms. The reason for this greater strain diversity at slaughter is unknown but may reflect strains being introduced to the flock by contaminated equipment or personnel when birds are collected and transported for slaughter. The Penner subtypes most frequently detected in this survey were also commonly associated with human gastroenteritis (Patton and Wachsmuth 1992).

Bird age is known to be strongly associated with campylobacter infection. The confounding effect of age was minimised by restricting the survey to birds of a single age. In the population studied, it was found that 50% of broiler flocks were infected with *C. jejuni* when birds were 5-6 weeks of age. This compared with a reported flock prevalence of infection of 76% by the time birds reached slaughter weight in a different broiler company in England (Humphrey and others 1993). Some of the flocks in the present study which were campylobacter culture-negative at 5-6 weeks of age were shown to be infected with *C. jejuni* at slaughter and this was most likely to reflect late acquired infection. Broiler houses were usually depopulated over a number of days and campylobacter infection of birds at slaughter was shown to be dependent on the stage of depopulation of the house; the first batch of birds slaughtered was less likely to be infected than subsequent batches. Although this may be purely associated with the age of the birds other factors may be hypothesised to be involved. For example, the risk of infection to the remaining birds in the flock may be increased by the presence of processing plant personnel or equipment when birds were collected for slaughter. This hypothesis was supported by evidence from two independent studies of broiler poultry which reported that on some occasions flocks did not become infected with campylobacter until after the first birds had been collected for slaughter (Jacobs-Reitsma and others 1994, Berndtson and others 1996). It should, however, be remembered that half of the flocks in this study were infected prior to any site depopulation and therefore infection could not have been acquired from this potential source.

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CHAPTER 6

A cross-sectional survey of thermophilic campylobacter infection of broiler flocks in England and Wales.

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Summary

A cross-sectional survey of 151 integrated broiler flocks, surveyed when the chicks were at least 5 weeks old, was conducted to estimate the prevalence of infection with thermophilic campylobacters and identify risk factors for infection. Sixty eight flocks (45%) were colonized with campylobacters, mainly *C. jejuni*. Therefore, the prevalence of campylobacter infection in flocks of housed broiler chicken in England and Wales during the finishing stages of production was estimated to lie between 36.9% and 53.1% with 95% confidence. The majority of birds in most infected flocks were shedding campylobacters.

Questionnaire data were analyzed using logistic regression to identify management and other factors significantly associated with campylobacter infection ($p \leq 0.05$). The risk of infection was increased by a high rodent population on the site (odds ratio = 6.82, $p = 0.003$), if the broiler house was not blown to remove debris during cleaning (OR = 67.79, $p = 0.005$), when the flock was close to another poultry site (OR = 2.48, $p = 0.038$), when drinking water was supplied to the birds by communal bell drinkers (OR = 41.18, $p < 0.001$) and when the broiler house fabric or equipment required repair (OR = 27.36, $p = 0.026$). It was estimated that together these factors accounted for approximately half of all campylobacter infections of broiler flocks in England and Wales at the finishing stages of production.

Introduction

Broiler flocks are frequently infected with campylobacters, mainly *C. jejuni* (Prescott and Munroe 1982, Tauxe and others 1985, Hood and others 1988, Humphrey and others 1993) and the consumption or handling of chicken has been considered a major risk factor for human campylobacteriosis (Harris and others 1986, Deming and others 1987). Infection of poultry is not generally associated with clinical illness even though large numbers of campylobacters are excreted in the faeces. *C. jejuni* colonizes the lower gastro-intestinal tract where the organism localises principally in the mucus layer of caecal and cloacal crypts with no evidence of pathological change (Doyle 1991). Campylobacter infection of poultry is known to be related to age (Smitherman and others 1984, Engvall and others 1986, Hoop and Ehram 1987). Infection is rarely detected in chicks which are less than one week old, flocks usually becoming infected when the birds are 3-5 weeks of age. Infection spreads rapidly through the flock and birds remain infected at least until slaughter at 6-7 weeks of age. The prevalence of campylobacter infection in broiler flocks in Great Britain has not been established and the epidemiology of infection in poultry requires further investigation (Evans 1992). In particular, the source or sources of infection remain unknown. Vertical transmission of campylobacters from infected breeder flocks and transmission in poultry feed have not been shown to be routes of infection (Shanker and others 1986, Humphrey and others 1993).

Hypothesised sources of campylobacter for broiler flocks include contaminated drinking water (Pearson and others 1993, Kapperud and others 1993) or fomites including personnel (Hoop and Ehram 1987, Annan-Prah and Janc 1988, Kazwala and others 1993, Humphrey and others 1993) and infected livestock or free-living animals (Annan-Prah and Janc 1988, Kapperud and others 1993). The ultimate aim must be to produce birds free from infection at slaughter and so reduce the potential for human infection from this source. The identification of risk factors for infection may allow the development of farm interventions to achieve this aim. The present study was conducted to identify potential risk factors for broiler flock infection with thermophilic campylobacters in England and Wales.

Materials and methods

Data collection

A cross-sectional survey of integrated commercial broiler flocks in England and Wales was conducted to estimate the prevalence of infection with thermophilic campylobacters and to identify risk factors for flock infection. All integrated broiler producing companies with flocks in England and Wales were approached and asked to participate in the survey. Nineteen companies agreed to be involved. These companies were responsible for over 75% of table chicken production in England and Wales. Each company was asked to survey approximately 20% of their company-owned sites and 20% of broiler production sites which were independently owned, but were contracted to the company. In order to limit the confounding effect of age and to avoid any risk associated with abattoir personnel or equipment visiting the site, companies were asked to recruit sites into the study when birds in at least one poultry house on the site were five weeks of age or older but, if possible, before any birds from the current production cycle had been slaughtered. Each site could only enter the study once. A relatively constant laboratory throughput was maintained by the provision of a timetable to each company for sampling. As a consequence, sites were randomly included in the study based on the sampling timetable and bird age requirements.

Data collection was carried out by the author or by company personnel who were familiar with the sites and the study protocol and experienced in obtaining cloacal swabs (Appendix B.1). Sixteen cloacal swabs were collected from birds in one randomly selected broiler house, containing birds which were at least 35 days of age, on each site on a single occasion. Birds were sampled at equal intervals from throughout the poultry building. The swabs were placed in Amies transport media with charcoal, labelled, packaged and posted to the laboratory. A standard questionnaire was completed for each site at the time of sampling (Appendix B.2). The questionnaire sought information on potential risk factors and confounders associated with campylobacter infection. It was divided into three sections:-

1. Site management and disease security precautions;

2. Demographic details of birds in each house on the site;
3. Details relating to the building and equipment, disinfection regimes and pest control used in the sampled house, and production data for these birds.

Microbiological methods

Cloacal swabs were cultured for campylobacter using an enrichment step and selective agar, an incubation temperature suitable for the isolation of thermophilic campylobacters and a micro-aerophilic environment. Two laboratories were used for the bacteriological examination and therefore "laboratory" was included in the analysis as an *a priori* confounder. The laboratory protocols used for the isolation and confirmation of thermophilic campylobacters have been described previously (chapter 5, Bolton 1985). A maximum of five isolates per site were speciated (*C. jejuni*, *C. coli*, *C. lari*) using recommended techniques (Goossens and Butzler 1992).

Statistical analysis

A flock of birds in an investigated broiler house was classified as infected, or positive, if one or more cloacal swabs yielded campylobacters and non-infected, or negative, if all swabs were negative. The campylobacter flock status was used as the outcome variable in the risk factor analysis (irrespective of campylobacter species). Questionnaire data were analyzed to identify management and other factors significantly associated with campylobacter infection.

Initially all variables were examined individually for association with campylobacter infection in a univariate analysis. Those variables associated with infection at $p \leq 0.20$ and all *a priori* confounders (laboratory, bird age and visits to site by abattoir personnel for the collection of birds for slaughter), irrespective of statistical significance, were modelled together in a single multi-variable regression model using the statistical package EGRET (Anon 1993b) in order to identify factors which were independently associated with infection. Variables were removed individually from the model constructed starting with the least significant until all variables remaining in the model were significant at $p \leq 0.05$. The model was then extended to include each previously excluded variable one at a time and any with a significant likelihood

ratio test statistic ($p \leq 0.05$) or which changed the estimated coefficients of one or more of the main risk variables by an appreciable degree were added to the model. As a final check all variables which were not in the original model were examined one by one in the same manner. Two-way interactions between model variables were tested and included if significant at $p \leq 0.05$.

The population attributable risk (PAR) was estimated for each of the identified risk factors using the method of Bruzzi and others (1985) and adjusted relative risks calculated by stratifying by all other risk factors. A summary PAR was calculated for all main risk factors acting together.

The probability of misclassifying the campylobacter status of flocks, with the sampling strategy used in this survey, was estimated by fitting a beta distribution to the proportion of infected birds in the surveyed flocks and using a Fortran programme to estimate the parameters of the beta distribution and their 95% confidence region. A threshold level of 5% was used for classification purposes (flocks with more than 5% of birds infected were classified as positive).

Results

Descriptive analyses

Data collection for the national survey commenced in September 1993 and was completed in April 1994 and involved 151 flocks on individual broiler sites. The sites involved in the survey produced 16.8 million birds for slaughter in the production cycle surveyed and annually produced 84 million birds (based on 5 production cycles per year).

The regional distribution of the broiler sites surveyed was: south east England (27%), south west England (15.2%), East Anglia (20%), West Midlands (15.2%) and Wales (3.3%). The nineteen participating companies managed between 1 and 100 flocks (mean = 36.5). The surveyed sites housed 9,000 to 360,000 broiler chicken (mean = 111,020, standard deviation = 85,451). The number of poultry houses on sites

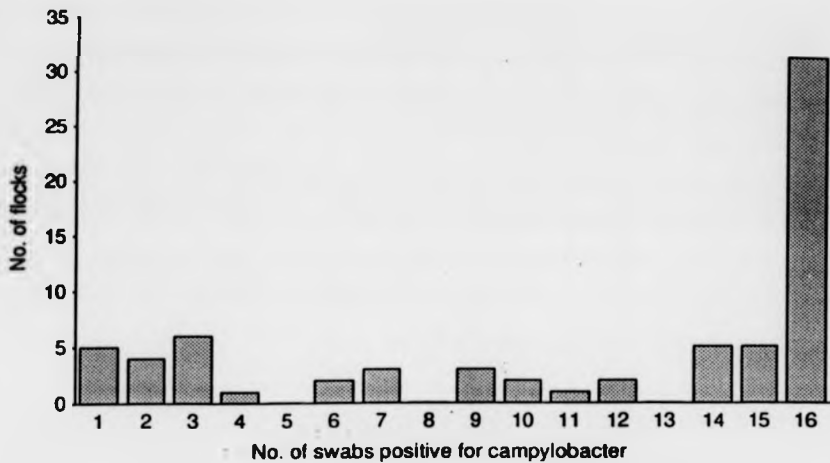


Figure 6.1. Distribution of campylobacter positive swabs in positive flocks

ranged from 1 to 14 and the number of birds within a sampled house from 4,000-60,000 (mean = 21,845, s.d = 10,398).

The age restriction incorporated in the study design ensured that most sites (67.6%) were surveyed when the birds were between 35 and 38 days of age and were due to be slaughtered within a few days. However, a small number of flocks (8.6%) were surveyed when the chicks were slightly younger in order to avoid sampling birds in houses which had been partially depopulated. Some older flocks, with birds up to 55 days of age, were also sampled reflecting the practice of heavy broiler (cockerel) production. There was a fairly wide distribution of chick ages on some of the larger sites and on 23 sites (17%) it was not possible to survey the site before any birds had been slaughtered. However, in all cases, none of the birds in the individual houses surveyed had been slaughtered.

Birds in 68 broiler houses (45%) were found to be shedding thermophilic campylobacters. Therefore, the prevalence of infection when birds were

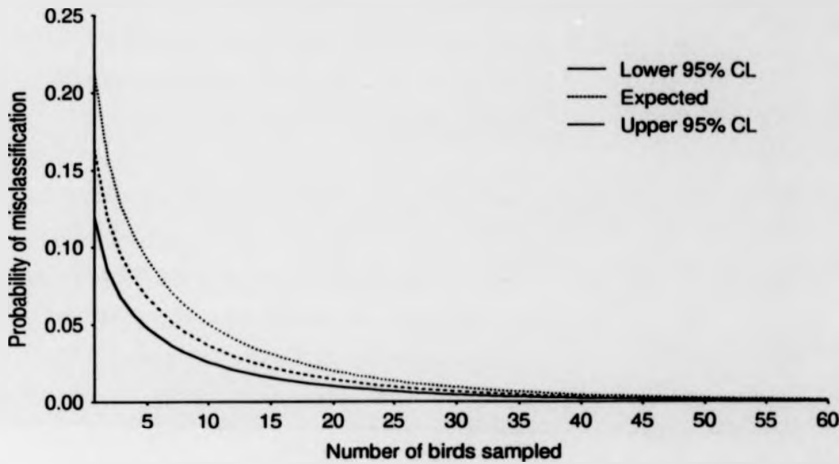


Figure 6.2. Probability of failure to detect infected birds by number of birds sampled among flocks with at least 5% of birds infected with campylobacter

approximately 5-6 weeks of age (mean = 37 days, s.d = 3.87 days) in flocks of housed broiler chicken in the studied population was estimated to lie between 36.9% and 53.1% with 95% confidence. *C. jejuni* was isolated from over 95% of positive sites and comprised 84% of speciated isolates. However, in 15% of positive sites other campylobacter species were detected (*C. coli* or *C. lari* or both) in addition to *C. jejuni* and in less than 5% of positive sites only non-*C. jejuni* species (*C. coli* or *C. coli* and *C. lari*) were detected.

Figure 6.1 shows the distribution of the number of campylobacter positive cloacal swabs out of the 16 examined from each site. Campylobacters were isolated from at least 14 swabs in 60.3% of positive farms. An estimate was made of the probability of misclassifying a flock, containing at least 5% of birds infected with campylobacter, when 16 birds were sampled per flock. The maximum probability of misclassification

over the 95% confidence region was 0.028 (2.8%). That is, in the long run, no more than 1 in 36 flocks with more than 5% of birds infected were likely to have been wrongly classified as campylobacter-negative by the sample size used in this survey for the detection of flock infection. Figure 6.2 shows the probability of failing to detect infected birds in flocks, where the prevalence of campylobacter infection is at least 5%, for a range of sample sizes.

Broiler production systems in England and Wales were highly standardised. The use of disease prevention measures varied very little in the studied population. For example, all sites operated an all-in, all-out stocking policy and the majority (96.7%) housed birds in controlled ventilation broiler houses of fairly standard construction. It was therefore not possible to examine the effects of site stocking policy or type of housing on the risk of campylobacter infection. Only factors for which there was sufficient variation in the population could be examined as potential risk factors for campylobacter infection.

Univariate analysis

Table 6.1 gives the results of the univariate analysis of risk factors for broiler flock infection with thermophilic campylobacters. The table lists all variables found to be associated with campylobacter infection at $p \leq 0.20$ and the variables which were considered to be *a priori* confounders. Eight factors out of the 61 variables examined were found to be associated in the univariate analysis with a risk of campylobacter infection at the 5% level of significance ($p \leq 0.05$). A further 17 factors were found to be associated with infection at the 5-20% level of significance ($0.05 < p \leq 0.20$).

All other variables examined were not associated with campylobacter infection at this level of significance. These were: farm location, broiler company size, feed mill ownership, flock ownership, person conducting survey, hatchery size, breed of chicks, month of sampling, staff contact with other birds, presence of other livestock, dogs or cats on site, presence of a site perimeter fence, presence of a warning notice at site entrance, use of a vehicle wheel bath, availability of written hygiene rules, availability of hand washing facilities, waterproof footwear worn, house wall

construction, drinking water source, feeding system, litter condition, litter disposal method, dead bird disposal method, wild bird access to poultry houses, rodent control programme operator, house fogging, house fumigation, insecticide usage, house empty period between production cycles, visit by vet, visitor hygiene score, therapeutic antibiotic usage, bird stocking density and age adjusted mortality percentage.

The 13 sites with at least some birds infected with *C. coli* or *C. lari* were examined to see if they differed in any significant way ($p \leq 0.05$) from sites where *C. jejuni* was the only detected species. There was an association with the presence of livestock (cattle, sheep, pigs or equines) on the site and the type of campylobacter species isolated; infections with *C. coli* or *C. lari* were more likely on sites where other livestock were present (OR=5.25, $p=0.013$). Non-*C. jejuni* infections were also more likely if dead birds were disposed of on the farm rather than off the site (OR=7.45, $p=0.006$), if feed was supplied by independently owned rather than broiler company owned feed mills (OR=5.00, $p=0.023$) and if the site was supplied with water from a borehole rather than mains water (OR=5.26) although this was not significant at this level ($p=0.079$). *C. jejuni* only infections were more likely if staff had contact with other birds (OR=9.29, $p=0.023$). These univariate associations could not be investigated further due to the paucity of data. Sites infected with different species of campylobacter did not differ in any other major respects.

Table 6.1. Univariate analysis - unadjusted odds ratios for campylobacter infection of broiler flocks ($p \leq 0.20$ and *a priori* confounders)

Exposure	All flocks (% exposed)	Positive flocks	Negative flocks	Unadjusted OR	p-value
Demography					
Company result (% of flocks infected)					
<33.3	72 (47.7)	19	53	1.000	<0.001
33.3-66.6	51 (33.8)	26	25	2.901	
>66.6	28 (18.5)	23	5	12.830	
(Test for linear trend: $\chi^2=26.107$ $p < 0.001$)					
Flock size (no. of birds)					
<50,000	32 (21.2)	15	17	1.00	0.108
50,000-99,999	56 (37.1)	26	30	0.982	
100,000-149,999	31 (20.5)	18	13	1.569	
$\geq 150,000$	32 (21.2)	9	23	0.444	
No. of birds in sampled house					
<10,000	21 (13.9)	14	7	1.000	0.177
10,000-19,999	47 (31.1)	20	27	0.370	
20,000-29,999	54 (35.8)	21	33	0.318	
$\geq 30,000$	29 (19.2)	13	16	0.406	
No. of staff (part time = 0.5)					
0.5-1	58 (38.4)	27	31	1.000	0.057
1.5-2	71 (47.0)	36	35	1.181	
>2	22 (14.6)	5	17	0.338	
Age of birds (days)					
<34	13 (8.6)	5	8	1.000	0.537
35-38	102 (67.6)	44	58	1.214	
>38	36 (23.8)	19	17	1.788	
(Test for linear trend: $\chi^2=1.17$ $p=0.279$)					

Table 6.1. (continued)

Exposure	All flocks (% exposed)	Positive flocks	Negative flocks	Unadjusted OR	p-value
Laboratory					
A	86 (57.0)	34	52	1.000	0.118
B	65 (43.0)	34	31	1.677	
Disease Security					
Proximity to other poultry sites					
>2 km	85 (56.3)	32	53	1.000	0.039
≤2 km	66 (43.7)	36	30	1.987	
No. of entrances to site					
One	111 (73.5)	46	65	1.000	0.140
>One	40 (26.5)	22	18	1.727	
Disinfectant boot dip					
No	9 (6.0)	6	3	1.000	0.178
Yes	142 (94.0)	62	80	0.388	
Concrete apron around house					
No	139 (92.1)	65	74	1.000	0.135
Yes	12 (7.9)	3	9	0.380	
House floor construction					
Concrete	138 (91.4)	59	79	1.000	0.066
Other	13 (8.6)	9	4	3.013	
Age of poultry house (yrs)					
≤4	23 (15.2)	5	18	1.000	0.012
>4	128 (84.8)	63	65	3.489	

Table 6.1. (continued)

Exposure	All flocks (% exposed)	Positive flocks	Negative flocks	Unadjusted OR	p-value
House or equipment in need of repair					
No	66 (43.7)	23	43	1.000	0.026
Yes	85 (56.3)	45	40	2.103	
Sanitizer in header tanks					
No	83 (55.0)	43	40	1.000	0.064
Yes	68 (45.0)	25	43	0.541	
Watering system					
Cup/nipple drinkers only	75 (49.7)	29	46	1.000	0.118
Bell drinkers	76 (50.3)	39	37	1.672	
Litter type					
Shavings, paper/other	98 (64.9)	50	48	1.000	0.043
Straw	53 (35.1)	18	35	0.494	
Litter beetles					
Light/no infestation	105 (72.9)	44	61	1.000	0.121
Some/heavy infestation	39 (27.1)	22	17	1.794	
Frequency live/dead rodents seen					
<weekly	122 (80.8)	47	75	1.000	<0.001
≥weekly	29 (19.2)	21	9	4.189	
Broiler building cleaning and disinfection regime					
Blown					
Yes	114 (75.5)	44	70	1.000	0.005
No	37 (24.5)	24	13	2.933	

Table 6.1. (continued)

Exposure	All flocks (% exposed)	Positive flocks	Negative flocks	Unadjusted OR	p-value
Steam cleaned					
Yes	12 (7.9)	3	9	1.00	0.135
No	139 (92.1)	65	74	2.632	
Site visitors since chick delivery					
No. of feed deliveries					
≤10	57 (40.7)	31	26	1.000	0.103
11-20	45 (32.1)	20	25	0.671	
21-30	23 (16.4)	7	16	0.367	
≥31	15 (10.7)	4	11	0.305	
(Test for linear trend: $\chi^2=5.824$ p=0.016)					
Visit by catchers for slaughter					
No	112 (83)	48	64	1.000	0.114
Yes	23 (17)	14	9	2.074	
Production data					
Antibiotic treatment					
No	118 (86.1)	56	62	1.000	0.078
Yes	19 (13.9)	5	14	0.395	
Disease diagnosed					
No	102 (67.6)	42	60	1.000	0.170
Yes	49 (32.4)	26	23	1.615	
Weight of birds					
≤ average for age	80 (53.0)	32	48	1.615	
< average for age	71 (47.0)	36	35	1.543	

Multivariable analysis

A final model of eight statistically significant ($p \leq 0.05$) independent risk factors for campylobacter infection of broiler flocks was formed using the regression modelling strategy described (table 6.2). The variables that were included in the model were the distance to the nearest poultry site, the type of drinker supplying water to the birds, the frequency with which rodents were seen on the site, whether the house was blown during clean out between production cycles, the state of repair of the poultry house and equipment, the laboratory conducting the bacteriology, the age adjusted bird mortality and the hatchery size. Flock size, company size and the type of feeding system, although non-significant in the model ($p > 0.05$), were also included because they altered the odds ratios of the main risk factors appreciably. Two of the risk factors (the type of drinker and whether the house was blown during cleaning) were found to interact statistically with the state of repair of the poultry house and equipment. The effects of these interactions are shown in table 6.3.

The variation explained by the regression model as measured by the square of the Pearson correlation between the binary outcome and the predictor was 44%. The population attributable risk per cent was estimated for each of the main risk factors. In descending order of importance, 15.4% of campylobacter infections were attributable to a rodent density on the site associated with mice or rats being seen at least once weekly, 15% to the house not being blown to remove debris between production cycles, 12.5% to the presence of a neighbouring poultry site within 2 kilometres, 11.8% to the use of communal bell drinkers and 6.6% to the recognized need for repair to the fabric or equipment of the broiler house building. The total amount of campylobacter infection attributable to exposure to all these risk factors acting together was estimated to be 49%.

Table 6.2. Multivariable analysis of risk factors for campylobacter infection of broiler flocks

Exposure	Campylobacter:		Adjusted OR	95% CL
	positive	negative		
Distance to nearest poultry site				
> 2 km	32	53	1.00	
≤ 2 km	36	30	2.48	1.01-6.08
Frequency rodents seen on site				
< weekly	47	75	1.00	
≥ weekly	21	8	6.82	1.70-27.33
State of repair of poultry house and equipment				
No repairs required	23	43	1.00	
Some repairs required	45	40	27.36	3.65-204.8
Type of watering system in house				
Cup or nipple drinkers	29	46	1.00	
Bell drinkers or mixed systems	39	37	41.18	6.40-264.8
House interior blown at last cleaning				
Yes	44	70	1.00	
No	24	13	67.79	7.40-621.0
Laboratory				
A	34	52	1.00	
B	34	31	4.14	1.04-16.54
Flock size (no. of birds)				
<50,000	15	17	1.00	
50,000-99,999	26	30	1.54	0.44-5.34
100,000-149,999	18	13	4.83	1.04-22.38
>150,000	9	23	0.97	0.19-4.81

Table 6.2. (continued)

Exposure	Campylobacter:		Adjusted OR	95% CL
	positive	negative		
No. of flocks managed by company				
≤25	10	9	1.00	
26-49	23	27	0.16	0.02-1.06
50-74	16	19	0.56	0.09-3.42
≥75	19	28	0.41	0.06-3.04
Age-adjusted mortality % (35 days)				
< 3	26	31	1.00	
3-3.99	20	23	1.08	0.36-3.25
4-4.99	10	10	0.66	0.14-3.04
>5	12	19	0.13	0.03-0.57
Hatchery incubator size (no. of eggs)				
<250,000	4	3	1.00	
250,000-499,000	7	14	0.21	0.02-2.47
500,000-749,000	16	19	1.41	0.15-13.60
750,000-999,000	31	32	2.25	0.23-21.86
>1000,000	10	15	0.34	0.03-3.69
Type of feeding system				
Pan or hopper feeders	19	28	1.00	
Chain feeders or mixed systems	49	55	0.36	0.09-1.33
Interaction of repair with drinker			0.03	0.003-0.28
Interaction of repair with blown			0.04	0.003-0.55

Table 6.3. Multivariable analysis - effects of interactions between risk factors**6.3a. Type of drinker and the state of repair of the poultry building**

Exposure	Campylobacter:		Adjusted OR	95% CL
	positive	negative		
Cup or nipple drinkers, no repairs required	10	34	1.00	
Bell drinkers and repairs required	26	28	33.53	4.99-225.3
Bell drinkers and no repairs required	13	9	41.18	6.40-264.8
Cup or nipple drinkers and repairs required	19	12	27.36	3.65-204.8

6.3b. Building blown to remove debris at cleaning and the state of repair of the poultry building

Exposure	Campylobacter:		Adjusted OR	95% CL
	positive	negative		
Blown during clean out, no repairs required	13	38	1.00	
Not blown during clean out, repairs required	14	8	77.51	7.32-820.3
Not blown during clean out, no repairs required	10	5	67.79	7.40-621.0
Blown during clean out, repairs required	31	32	27.36	3.65-204.8

Discussion

The sites involved in the survey annually produced about 20% of all chicken slaughtered in England and Wales. The regional spread of surveyed sites compared reasonably well with the regional distribution of all sites with over 1,000 birds (June 1993 Agricultural Census, MAFF) except for a slight bias in the study towards the south east England which was due to the fact that the pilot study was conducted in this region. There was a slight bias towards company owned or managed sites in the survey; participating companies owned or managed 45% of their associated sites, but in the survey these represented 59% of sites. The average flock size of investigated flocks was much larger than in the general population. In the general population over 50% of sites contain less than 1000 birds (June 1993 Agricultural Census, MAFF), but together these account for only a very small percentage of chicken production. Therefore, these small sites were not included in the surveyed population. The method of site selection within broiler companies, although not truly random, was unlikely to be subject to any appreciable degree of bias. Therefore, it was considered that the results of the survey could be generalised to the entire population of integrated commercial broiler flocks in England and Wales.

It was estimated from this survey that between 37% and 53% of housed broiler flocks in England and Wales were infected with thermophilic campylobacters at the finishing stages of production. However, the colonization rate at slaughter was likely to be greater as a proportion of flocks may become infected during depopulation of the site (chapter 5, Jacobs-Reitsma and others 1994). This is the first campylobacter prevalence survey reported for England and Wales and there have been relatively few surveys conducted in other countries. However, recent surveys in The Netherlands and Norway reported that 82% of 187 and 18% of 176 investigated broiler flocks respectively were infected with campylobacters at slaughter (Jacobs-Reitsma and others 1995a, Kapperud and others 1993). A recent study of a limited number of broiler flocks in Sweden found 27% of flocks and 39% of production cycles were infected with campylobacter in a one year study period but 16/18 of the sites (89%) housed infected birds in at least one production cycle during the year (Berndston and others 1996a). Production systems in England and Wales more closely resemble

systems in The Netherlands than Scandinavia, where poultry management is less intensive. Therefore, the estimated campylobacter prevalence in British broiler flocks was not unexpected.

As anticipated from the results of the pilot study, infection appeared to be an all or nothing event with most birds in an infected flock shedding campylobacters. Therefore, the probability of misclassifying a truly infected flock is small even if only a small number of birds are sampled in investigated flocks. In fact, the probability of failing to detect infected birds when at least 5% of birds in the flock are infected with campylobacter ranges from 1.4-2.8% when 16 birds are sampled from the flock (therefore, a maximum of 4 flocks could have been misclassified by this survey). This level of misclassification is unlikely to bias the results. For the purposes of efficiency in future studies, a range of probabilities of misclassification were calculated for varying numbers of sampled birds. For example, sampling 14 birds per flock will misclassify less than 3.5%, 22 birds less than 2% and 30 birds less than 1% of flocks in this way. Sampling greater than 30 birds per flock is inefficient as the probability of misclassification reduces very little above this sample size.

The main species of thermophilic campylobacter detected in broiler chicken was *C. jejuni* and this is in agreement with other studies (Engvall and others 1986, Kapperud and others 1993, Humphrey and others 1993). *C. coli* or *C. lari* were detected in some or all of the sampled birds on 15% of sites. However, the prevalence of these other species may have been underestimated due to the small number of birds sampled in each flock. The univariate analysis notably associated *C. coli* or *C. lari* infections with the presence of livestock on the site and a borehole water supply. *C. coli* is common in livestock, especially pigs, and un-disinfected water is a possible environmental reservoir of campylobacter. Therefore, although it was not possible to analyze species-specific risk factors in the multivariable model, these exposures may be hypothesised to be risk factors for infection with *C. coli* or *C. lari*.

The confounding effect of bird age and the potential confounding effect of slaughterhouse personnel visiting the sites appeared to be adequately controlled for

in the study design as neither variable was significantly associated with campylobacter infection in the multivariable analysis. The laboratory conducting the bacteriology had a small effect on the risk of infection but this was more likely to be due to the laboratory acting as a marker for other variables such as season, broiler company and region rather than a difference in the sensitivity of the methods of campylobacter detection. This survey was restricted to the winter months and no effect of season was apparent. This does not preclude a summer-winter seasonality which has previously been reported (Jacobs-Reitsma and others 1994).

The risk factors for campylobacter infection of broiler flocks identified in this survey were:-

1 The presence of a rodent density on the site associated with seeing live or dead mice or rats at least once per week increased the risk of infection (OR=6.82, 95% CL: 1.70-27.33, PAR=15.4%). This was in agreement with other studies (Kasrazadeh and Genigeorgis 1987, Annan-Prah and Janc 1988, Kapperud and others 1993). It was not possible to differentiate this risk between mice and rats due to sparse data.

2 The risk of infection was increased if the house was not blown to remove dust and debris during the clean out procedure between production cycles (PAR=15%). This risk was modified by the state of repair of the house. The highest risk occurred when the house was not blown during clean out and repairs were required (OR=77.51, 95% CL: 7.32-820.3). The risk was slightly lower if the house was not blown but repairs were not required (OR=67.79, 95% CL: 7.4-621) but the confidence limits overlapped considerably. The presence of organic matter can limit the effectiveness of disinfectants and therefore allow campylobacters to survive to infect subsequent flocks. Removal of organic matter by procedures such as sweeping, vacuuming and blowing the house are consequently likely to reduce the risk of this carry over of infection.

3 The presence of another poultry site within 2 kilometres of the site increased

the risk of infection (OR=2.48, 95% CL: 1.01-6.08, PAR=12.5%). It can be hypothesised that this may have been due to horizontal transmission of infection between sites by wildlife reservoirs or movement of vehicles, personnel etc. This was in agreement with the Swedish study of campylobacter infection of broiler flocks (Berndtson and others 1996a).

4 Some or all of the birds drinking water supplied by communal bell drinkers rather than individual cup or nipple drinkers increased the risk of infection (PAR=11.8%). The effect of the type of drinker was also modified by the state of repair of the poultry house or equipment. The highest risk involved the use of bell drinkers when no repairs were required (OR=41.18, 95% CL: 6.40-264.8). A slightly lower risk occurred when bell drinkers were used and repairs were required (OR=33.53, 95% CL: 4.99-225.3) although confidence limits again overlapped. The drinking water has been hypothesised previously to be a source of campylobacter infection (Pearson and others 1993, Kapperud and others 1993). However, it is not clear whether water is a primary source or simply a potent route of transmission of infection within flocks. The majority of farms (90.1%) in the survey were supplied with mains water which, due to chlorination, should be free of campylobacter at source. The large difference in risk evident between the two types of drinking systems was still apparent if the analysis was restricted to mains water users only. This indicates that water was unlikely to be a primary source of infection. However, the water in open communal bell drinkers may have been a) more likely to become contaminated with campylobacters (Jacobs-Reitsma and others 1994a) presumably from sources within the house and due to the inactivation of chlorine by a build up of organic matter in the drinkers and b) responsible for rapidly spreading the organisms between birds especially as palatine carriage of campylobacters by poultry has been reported (Montrose and others 1985, Shanker and others 1990). Following the introduction of infection, campylobacters would be detected earlier in birds using bell rather than cup or nipple drinkers due to the dependency of the rate of transmission of infection within the flocks on the type of drinker used. Therefore, in this age restricted survey bell drinkers appeared to increase risk of infection.

5 The requirement for repairs to the poultry house or equipment increased the risk of infection (PAR=6.6%). As shown above this factor interacted with two other risk factors. However, the need for repairs alone, when cup or nipple drinkers were used and the house was blown during clean out, was also associated with an increased risk of infection (OR=27.36, 95% CL: 3.65-204.8). The state of repair of the house was closely correlated with the age of the house. It is plausible that older houses in a poor state of repair may be more difficult to adequately clean between flocks and more likely to allow access to potential vectors of infection such as rodents than new houses in a good state of repair.

The odds ratios associated with the hatchery size and the age adjusted mortality percentage were relatively small and non-linear and in most strata the confidence intervals crossed unity. These variables were mainly included in the model due to their effect on the odds ratios of the main risk factors. However, an association between the hatchery of origin of chicks and campylobacter infection of broiler flocks has been recently reported leading to the conclusion that it may not be possible to rule out vertical transmission as a possible route of campylobacter infection (Pearson and others 1996). This finding is contrary to most other published studies. There was no evidence that campylobacter infection adversely affected broiler production.

It is difficult to accurately calculate attributable risks from cross-sectional surveys as the odds ratio may not be a good estimate of the relative risk. Therefore, an estimate was made of the population attributable risks using summary relative risks calculated by stratifying by all other risk factors rather than using odds ratios generated by the multivariable analysis. Most sites were exposed to some but not all of the risk factors but sites which were not exposed to any of the five main risk factors were considerably less likely to be infected. Twenty two sites were not exposed to any of these risk factors and only two of these sites were infected with campylobacter (prevalence = 9.1%, 95% CL: 0-21.35%). It was estimated that together these factors accounted for approximately half of all campylobacter infections at this stage of production in the general population of integrated broiler flocks.

The cross-sectional approach has the limitation that exposure and disease status are only investigated at one point in time and therefore has limited ability to identify causal associations. In addition, the relatively small number of flocks investigated in this survey has resulted in odds ratios having very wide confidence limits and will not allow the detection of any factors which have only a small effect on risk. However, this study has highlighted risk factors for infection which together may account for a considerable proportion of campylobacter infection in commercial broiler flocks in England and Wales thereby providing the basis of a control strategy. Further studies are required to confirm these findings before specific interventions can be recommended but the following preventive measures are likely to have the greatest impact on reducing the prevalence of campylobacter infection in broiler flocks in England and Wales: a) adequate rodent control b) thorough cleaning of houses between production cycles c) adequate hygiene barriers with neighbouring poultry sites d) the use of individual cup or nipple drinkers and e) maintaining the broiler house and equipment in a good state of repair.

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CHAPTER 7

A longitudinal study of thermophilic campylobacter infection of poultry broiler flocks in Great Britain.

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Summary

A longitudinal study was carried out to investigate risk factors for campylobacter infection of poultry broiler flocks. One hundred flocks associated with 5 integrated poultry companies were monitored for one production cycle. Bacteriological samples were collected from one house of birds on each site and examined for the presence of campylobacter at weekly intervals from 3-4 weeks of age until the birds were infected with campylobacter or the flock was depopulated, whichever was sooner. Environmental samples were obtained from 20 houses after cleansing and disinfection of the site before chick arrival. Conventional methods were used for the isolation of campylobacter and isolates were stored for molecular typing. Questionnaires collected information on potential risk factors for campylobacter infection. Survival analysis was used to assess the influence of various exposures on the age at which the flock was infected with campylobacter, using three different modelling techniques (proportional hazards regression, log-normal regression and discrete time survival analysis). The prevalence of campylobacter infection was strongly associated with age. Forty per cent of flocks were infected by the time the chicks were 3 weeks of age and more than 90% by 7 weeks. It was shown that infection spread rapidly to the majority of birds in a flock. Infection was not predictable by campylobacter status of the last flock reared on the site. However, as most flocks were infected, the power to detect such an association was poor and molecular typing studies may be more conclusive. No true environmental survival was seen in broiler houses after adequate cleansing and disinfection. The three statistical models gave similar results but the discrete time model was considered most appropriate. The most important predictors of survival were related to effective hygiene barriers such as housing birds in buildings in a good state of repair, appropriate usage of disinfectant boot dips and a high standard of cleansing and disinfection of the drinking water equipment. There was no evidence that rodents were a source of infection but most sites operated effective vermin control programmes. It seems most likely that campylobacter infection is introduced sporadically to chicks from the external site environment, perhaps by poultry farm workers or wildlife vectors. Therefore interventions aimed at improving hygiene barriers are likely to reduce the incidence of campylobacter infection of broiler flocks.

Introduction

Thermophilic campylobacters are the most frequently isolated pathogens from cases of human gastro-intestinal disease in the United Kingdom. There were approximately 44,000 laboratory reports of these infections during 1995 in England and Wales. The most common species is *C. jejuni* which accounts for 90% of cases (Anon 1993c). Poultry are recognized as an important reservoir of infection. Broiler flocks are frequently infected with campylobacters, mainly *C. jejuni* (Prescott and Munroe 1982, Hood and others 1988, Humphrey and others 1993), and a number of studies have shown that the consumption or handling of chicken is a major risk factor for human campylobacteriosis (Harris and others 1986, Deming and others 1987).

A cross-sectional survey of broiler flocks in England and Wales found that 45% of flocks (95% CL: 36.9-53.1%) were colonized with campylobacter when the birds were approximately 5 weeks of age (chapter 6). The risk of infection was increased -

- by a high rodent population on the site;
- on sites where the broiler house was not blown to remove debris during cleaning;
- when the broiler site was in close proximity to other poultry sites;
- when drinking water was supplied by communal rather than individual drinkers;
- when the broiler house fabric or equipment required repair.

A small number of the flocks in the survey, which were free of infection at five weeks of age, were screened again when the birds were slaughtered. It was found that some of these flocks were infected with campylobacter and infection was related to the stage of depopulation of the broiler house but it was not clear whether this was an age dependent effect or due to the potential risk of introduction of infection by contaminated equipment or personnel during the collection of birds for slaughter (chapter 5).

A longitudinal study was conducted to investigate the findings of the cross-sectional survey further. The objectives of the study were to investigate whether:-

- 1 Risk of campylobacter infection is dependent on bird age;
- 2 Rodents are a source of campylobacter infection for broiler flocks;
- 3 Carry over of infection to birds in successive production cycles occurs due to inadequate cleansing and disinfection of the broiler house;

- 4 The spread of infection within flocks is dependent on the type of drinker equipment;
- 5 The collection of birds for slaughter by processing plant vehicles, equipment and personnel increases the risk of infection for birds remaining on the site.

Materials and methods

Study population

The study population consisted of selected broiler production sites associated with five integrated poultry companies. Each company was involved in the previous cross-sectional survey and the companies were chosen for inclusion in the present study due to their large size and readiness to collaborate in the project. Together, the companies were responsible for about half of the table chicken produced in the United Kingdom.

Study design

In total, 100 broiler flocks were monitored longitudinally for the presence of campylobacter during one production cycle. Each broiler company was asked to recruit 20 broiler sites for the study (none of which were included in the previous cross-sectional survey).

Data collection

Flocks were regularly recruited into the study over a 12 month period (December 1994 to December 1995). Data collection was carried out by broiler company personnel or site owners/managers who were familiar with the study protocol and competent at obtaining cloacal swabs from live birds. One broiler house was studied on each broiler site and 16 cloacal swabs were obtained from birds within this house on the following occasions:-

- 1 At final depopulation of the flock in the study broiler house (Appendix C.1);
- 2 Birds in the next production cycle, housed in the study broiler house, were swabbed at weekly intervals from 28 days of age to, and including, final depopulation of the flock or until the birds were shown to be infected with campylobacter, whichever was sooner (Appendices C.3 - C.4).

Environmental samples were collected on 20 of the broiler sites after cleansing and disinfection of the study house before the birds in the flock to be surveyed arrived (Appendix C.2). Amies transport swabs were used for obtaining the samples, as in the collection of samples from live birds, but were first moistened by dipping in sterile water. The protocol involved sampling 16 different areas in the house including the header tank, drinkers, wooden support posts, slave feed hoppers, walls and floor. Five of these sites were visited by a member of the study team for detailed bacteriological examination of the broiler house environment before and after cleansing and disinfection to validate the sampling protocol. Cloacal swabbing of birds on these 20 sites commenced when birds reached 21 days of age rather than 28 days.

Exposure variables

Exposure data were collected by questionnaires (Appendices C.5 - C.7) which recorded details of:-

- 1 Flock demography;
- 2 General site management and disease security;
- 3 Methods of cleansing and disinfection of the study broiler house and equipment;
- 4 Rodent presence in the study broiler house and feed store and methods of control;
- 5 Birds drinking water supply and type of drinkers in the study broiler house;
- 6 Slaughter timetable for birds in all broiler houses on the site.

The examined variables are described in more detail in table 7.1.

Microbiological methods

All samples were sent by post to the Bacteriology Department, Central Veterinary Laboratory. Conventional methods were used for the isolation of campylobacter (chapter 5). Isolates were speciated and stored for molecular typing by restriction fragment length polymorphism of polymerase chain reaction products (Ayling and others 1996).

Table 7.1. Exposure variables

Rodent presence	
Rodent score	- evidence of the presence of mice and rats assessed by scoring individual signs (droppings, holes, smears, urine pillows, damage and live or dead animals)
Rodent control	- Operator - baiting procedure (no. bait points, product, frequency of replenishment).

Cleaning and disinfection of broiler house	
Structure of house	- age, floor and wall construction, feeding and drinking systems concrete apron around house, waterproof electrics
Condition of house	- building/equipment repair requirement
Cleaning method	- method of dust removal, litter removal, cleaning, disinfection, fumigation, method of cleaning: header tanks, feed lines, store area, area outside house
Product usage	- product type, concentration, amount used, timing of application (detergent/sanitiser, disinfectant, fumigant)
Cleaning score	- subjective score for effectiveness of cleaning floor, beams, fans, feed hopper, anteroom
Empty period	- times between: slaughter of flock and repopulation, disinfection and litter placement, disinfection and repopulation
Use of boot dips	- product, concentration, frequency of replenishment, when and where used

Drinking water supply	
Water source	- mains, borehole etc
Type of drinker	- cup, nipple, bell
Water sanitiser	- product, concentration, when used

Campylobacter testing result of previous flock	
Age at slaughter	- days
Campylobacter result	- positive, negative

Table 7.1. (continued)

Slaughter timetable

Age of birds in study house when first birds from site slaughtered
Age of birds in study house when first birds from study house slaughtered

Miscellaneous variables

Domestic animals	- other livestock - dogs, cats
Disease security	- perimeter fence, proximity to other poultry sites, dead-bird and litter disposal, wash water disposal, staff hygiene precautions
In feed medication	- antibiotic usage and age of birds at withdrawal - growth promotant usage and age of birds at withdrawal - coccidiostat usage and age of birds at withdrawal

***A priori* confounding variables**

Season	- month flock was infected with campylobacter or censored
Poultry company	- associated parent broiler producing company
Flock size	- flock size, number of broiler houses on site, number of birds in study house

Statistical analysis

Survival analysis (Collett 1994) was used to assess the influence of various exposure variables on the age at which the flock became infected with campylobacter. This method of analysis was chosen because the incidence rate was known to vary rapidly over age and the majority of flocks were likely to become infected. The survival time of a flock was the age at which campylobacter infection was first detected. Some flocks remained free of campylobacter throughout their lifespan and a few flocks were lost to follow-up due to missed sampling. These incomplete observations, where it was known only that the flocks survived until they were last sampled, are

said to be censored and this type of analysis is able to cope with such censored values. Survival was measured at discrete times, as in most studies of non-lethal events, the weekly testing schedule meant that it was known only that infection occurred within a time interval. The five time periods used for the survival analyses were from 0 - 28 days and weekly thereafter until 56 days of age.

The distribution of survival times in a group of individuals can be represented in terms of the survivor function, $S(t)$. This is the probability of survival beyond time t and is given by $S(t) = 1 - \Pi(t)$ where $\Pi(t)$ is the probability that the event occurs by time t . A plot of the survivor function against time consists of a series of horizontal lines joined by descending vertical steps each time a flock is detected as infected with campylobacter. Survival is analogous to the more familiar epidemiological concept of cumulative prevalence. The survival experience may also be characterised in terms of the incidence rate of the event, $\lambda(t)$, known as the hazard rate. $\lambda(t)$ is the risk of infection within a short time period given that the flock has survived to time t . For data grouped into a series of time intervals the average hazard per unit time is estimated by the observed number of flocks becoming positive in that interval divided by the average time survived in that interval. A graph of the hazard function shows how the risk of infection changes over time and is used to detect when the event of interest is most likely to occur. Estimation of the survival and hazard functions and their confidence limits was by the life table method (Collett 1994).

Table 7.1 lists the exposure variables studied. The effect on survival of these various exposures can be expressed as the hazard ratio which is the ratio of the hazard of infection at any time for an individual at a particular level of the variable to the hazard for an individual at another level, usually taken to be the first exposure level.

The initial exploratory stage of the analysis assessed each variable individually for its association with survival. This was done using the log-rank test (Peto and Peto 1972) and Gehan's generalized Wilcoxon test (Gehan 1965) for binary variables and Mantel's procedure (Mantel 1967) for variables with more than two categories. A

proportional hazards regression (PHR) model (Cox 1972) was fitted in order to estimate the corresponding hazard ratios and to test continuous variables and time-dependent variables (exposures which may be related to survival but which change over time). Graphs of the Kaplan-Meier estimates of the survivor function were examined to assess informally the likely validity of the proportional hazards assumption. Survivor functions which do not cross each other support the assumption that the hazard of infection at any given time for a flock in one variable category is proportional to the hazard at that time for a similar individual in another category.

Factors which the single variable analysis suggested might be related to the age of infection were then examined jointly in a multivariable proportional hazards regression in order to establish which variables best predicted survival. Correlations were examined among the initially screened variables to control for multi-collinearity. The initial model contained all the variables found to be individually associated with survival by the tests described above at a significance level of $p < 0.30$. Variables were then discarded in turn from the model, using a backward elimination procedure based on the likelihood ratio test statistic (LRS), until all remaining variables were associated with survival at $p < 0.10$. Finally, all variables which were not in this model were tested individually for inclusion using the same significance level of $p < 0.10$ and any which altered the regression coefficients by more than 50% were noted. At all stages of this procedure variables with more than 10% of values unknown or missing or with an exposure of less than 10% were discarded. Interactions between model variables were not tested due to paucity of data.

The effect of a predictor can vary over time and when it does the proportional hazards assumption is violated. In order to formally test the validity of this assumption a new time-dependent covariate was formed for each variable in the model, which represented the interaction of time with the exposure. This allowed the situation where the hazard ratio varied over time to be modelled. A non-significant result for the effect of this new predictor suggested that the effect of the predictor was constant over time. If violation was detected, the interaction with time remained in the model to ensure the appropriate estimation of exposure effects.

The PHR model has flexibility and widespread applicability because it makes no assumptions about the form of the probability distribution of survival times. However, it may lack power to detect associations with survival in comparison with other methods based on the valid assumption of a particular probability distribution and therefore a log-normal regression model was fitted as an alternative (Cox and Oakes 1984). In order to investigate the effect of collecting survival data at intervals, survival was also modelled using a modification of logistic regression known as discrete time survival analysis which takes into account whether or not infection is detected on the different testing occasions (Collett 1994). Both of these alternative models used the same procedure for eliminating variables from the initial multi-variable model except that the selection of variables for inclusion was based on a significance probability of $p < 0.20$ in the corresponding univariate model.

Statistical analyses were performed using the statistical software packages STATISTICA 5.0 (Anon 1995), EGRET (Anon 1993b) and GLIM4 (Francis and others 1993).

Results

Of the 100 broiler sites studied, the campylobacter prevalence when birds were surveyed at slaughter was 81.6% but this was strongly dependent on age (table 7.2). In the subsequent production cycle, 91% of the flocks studied longitudinally became infected with campylobacter and again the prevalence of infection was associated with age (table 7.3). Eight of the 20 flocks which were tested when the birds were only 21 days old were infected with campylobacter. The prevalence of campylobacter infection within infected flocks was usually very high and on more than 80% of positive sampling occasions campylobacters were isolated from at least 15 cloacal swabs (figure 7.1). *C. jejuni* was most commonly isolated (88.8% of flocks) but occasionally *C. coli* (3.4%) was the only species detected. Some flocks (7.9%) had mixed infections. Results of molecular typing will be reported separately. Twenty broiler sites collected environmental samples after cleansing and disinfection of the broiler house but none of these yielded campylobacters. The in-depth bacteriological studies on 5 of these sites supported these findings. Despite potential niches for the

Table 7.2. Campylobacter prevalence at slaughter by age of birds

Age (days)	Campylobacter			Unadjusted
	Negative	Positive	(% positive)	OR
28-35	3	3	(50.0)	1.00
36-42	9	19	(67.9)	2.11
43-49	5	25	(83.3)	5.00
≥ 50	1	33	(97.1)	33.00
All flocks	18	80	(81.6)	

(Test for linear trend: $\chi^2 = 12.82$ $p = 0.0003$)

Table 7.3. Life table

Interval (age in days)	Number uninfected at start	Number censored	Number exposed	Number infected	Proportion infected	Cumulative proportion infected	Hazard rate
0-28	100	0	100	44	0.44	0.44	0.02
29-35	56	0	56	18	0.32	0.62	0.05
36-42	38	6	35	19	0.54	0.83	0.11
43-49	13	3	11.5	7	0.61	0.93	0.13
50-56	3	0	3	3	1.00	1.00	0.29

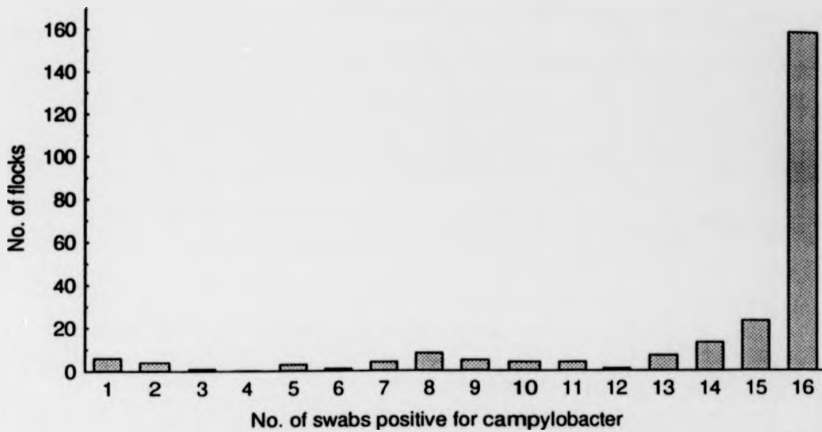


Figure 7.1. Distribution of campylobacter positive swabs in positive flocks (all samplings)

survival of campylobacter in moist litter trapped in various parts of the house, no true environmental survival within the broiler house was demonstrated after adequate cleansing and disinfection. However, on one site the plastic bell drinkers were not cleaned or disinfected and campylobacters were isolated from this equipment after the house had been disinfected.

The survival and hazard functions for the whole sample of flocks are displayed in figures 7.2 and 7.3 and the corresponding life table is given in table 7.3. A gradual increase in hazard rate over time was apparent which showed that the risk of infection increased with age to a peak at 50-56 days. However, caution is needed in interpreting this estimate since there were few events after 6 weeks of age.

A summary of the variables univariately associated with survival at $p \leq 0.30$ in a proportional hazards regression is shown in table 7.4. Hazard ratios exceeding unity indicate an increased hazard of infection and those less than unity a reduced hazard relative to the baseline category of the variable. The parent broiler company and the

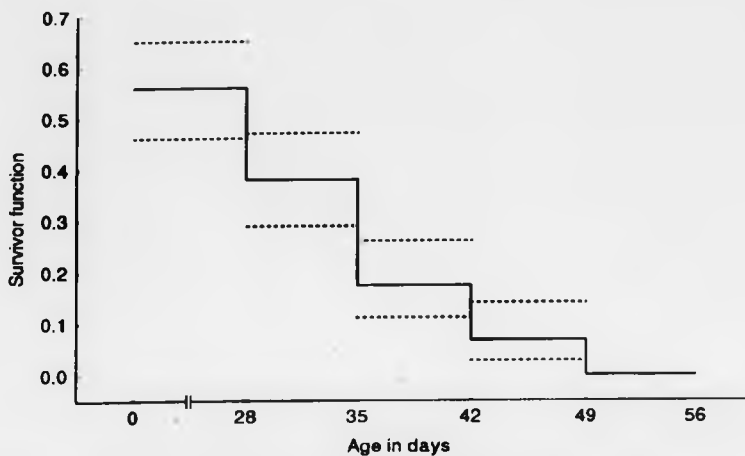


Figure 7.2. Survivor function and 95% confidence limits

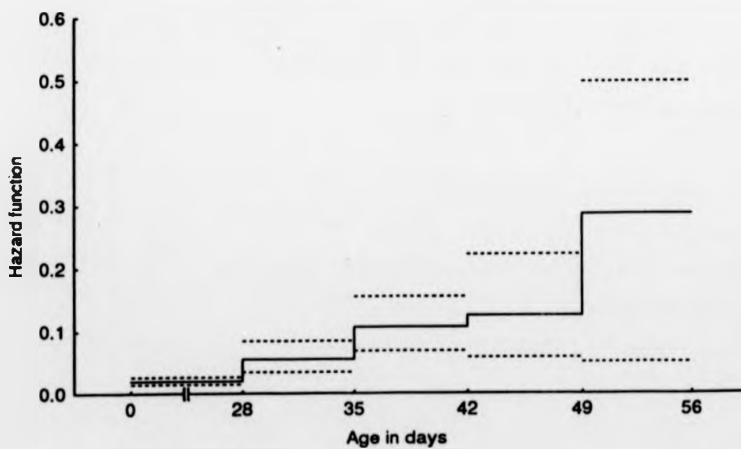


Figure 7.3. Hazard function and 95% confidence limits

season of the year were not associated with survival. Neither were any time varying variables, which were those whose value may change over time, (whether flock depopulation had commenced, whether in-feed medication was in use). Therefore, their results are not given in the table. The median survival time indicates the age at which at least 50% of the flocks were infected with campylobacter.

The results of the PHR multivariable analysis are shown in table 7.5. The model used information from 99 of the 100 flocks examined, a single flock being omitted due to missing data for an exposure variable. An increased hazard of infection was associated with the broiler house requiring structural repair, inadequate cleansing and disinfection of the water header tank, changing the disinfectant solution in the boot dips at broiler house entrances less than once a week and using a boot dip only after litter or chick arrival rather than after house disinfection or at all times. Survivor and hazard functions for these variables are plotted in figures 7.4a-7.7a and 7.4b-7.7b.

The effects of the variables in the PHR model did not vary over time as none of the interactions of the model variables with "time" were significant, the probabilities ranging from 0.26 to 0.89. This confirmed that the proportional hazards hypothesis was an acceptable assumption upon which to base the modelling.

The LNR multivariable model (table 7.6) included all the terms in the PHR model and two further variables, the number of houses on the site ($p=0.055$) and the type of feeding system ($p=0.031$). The regression coefficients indicated that a shorter survival time was associated with flocks with a larger number of broiler houses on the site and where a chain feeding system was used compared with a hopper/pan type.

The results for the final discrete time model (table 7.7) are based on a sample of 96 flocks and the variables included all those in the PHR model as well as an extra one which suggested that the removal of dead birds from the site lowered the hazard of infection compared with on-site disposal. The proportional hazards assumption also appeared to be satisfactory, none of the interactions of the model variables with time being significant with the probabilities ranging from 0.26-0.72.

Table 7.4. Initial selection of variables for proportional hazards regression

Exposure	n	p values:			Hazard ratio	Median survival (days)
		Wilcoxon	Logrank	PHR		
Rodent control						
Site staff	10	0.131	0.073	0.222	1.00	42
Contractor	90				1.51	35
Live or dead rats seen						
None	95	0.161	0.047	0.185	1.00	35
1-2	5				0.53	42
Bait product						
Poor	2	0.137	0.066	0.261	1.00	49
Effective	64				2.15	35
Feeding system						
Hopper/pan	47	0.028	0.152	0.285	1.00	35
Chain	53				1.25	28
Broiler house due for repair						
No	54	0.007	0.009	0.046	1.00	39
Yes	46				1.53	28
Equipment requires repair						
No	69	0.025	0.027	0.075	1.00	35
Yes	29				1.55	28
Broiler house cleaner						
Contractor	95	0.109	0.097	0.293	1.00	35
Farm staff	5				0.63	42
Method of dust removal at cleaning						
Swept/blown/washed	79	0.150	0.147	0.287	1.00	35
Blown and washed/other	21				0.76	35

Table 7.4. (continued)

Exposure	n	p values:			Hazard ratio	Median survival (days)
		Wilcoxon	Logrank	PHR		
Interval between washing and disinfection						
<24 hrs	59	0.142	0.151	0.279	1.00	35
≥ 24 hrs	37				1.27	28
Disinfectant						
Poor	26	0.150	0.106	0.241	1.00	32
Good	8				0.60	42
Boot dip						
Poor	29	0.173	0.160	0.294	1.00	35
Good	67				1.29	35
Dead bird disposal						
On site	33	0.340	0.126	0.252	1.00	35
Removed from site	64				0.77	35
Boot dip use						
At all times	68	0.015	-	0.097	1.00	35
After disinfection	17				0.56	42
After litter or chick arrival	15				1.13	28
Frequency boot dip replenished						
< once per week	17	0.075	0.073	0.143	1.00	28
≥ once per week	83				0.65	35
No. of birds on site (log)						
(Continuous variable)	99	-	-	0.189	1.18	-
No. of broiler houses						
(Continuous variable)	100	-	-	0.201	1.04	-
Method of cleaning header tank						
None/washed only	23	0.058	0.028	0.084	1.00	28
Disinfected/sanitised/new equipment	76				0.64	35

Table 7.5. Exposure variables in the final proportional hazards regression model

Exposure	Coefficient	Standard error	p-value (LRS)	Hazard ratio	95% CL
House repairs due	0.567	0.225	0.012	1.76	1.13-2.74
Header tank disinfected	-0.445	0.252	0.087	0.64	0.39-1.05
Boot dip changed \geq weekly	-0.560	0.295	0.070	0.57	0.32-1.02
Boot dips after house disinfection	-0.593	0.324	0.056	0.55	0.29-1.04
Boot dips after litter/chick arrival	0.326	0.305		1.39	0.76-2.52

Table 7.6. Exposure variables in the final log-normal regression model

Exposure	Coefficient	Standard error	t value	Probability
House repairs due	-0.365	0.110	-3.308	0.001
Header tank disinfected	0.211	0.123	1.723	0.069
Boot dip changed \geq weekly	0.378	0.142	2.653	0.009
Boot dips after house disinfection	0.357	0.150	2.387	0.019
Boodips after litter/chick arrival	-0.231	0.151	-1.537	0.128
Number of houses on site	-0.030	0.015	-1.939	0.055
Chain feeding system	-0.235	0.107	-2.192	0.031
Constant	0.597	0.200	2.982	<0.001

Table 7.7. Exposure variables in the final discrete time model

Exposure	Coefficient	Standard error	p-value (LRS)	Hazard ratio	95% CL
House repairs due	0.898	0.257	<0.001	2.45	1.48-4.06
Header tank disinfected	-0.662	0.272	0.020	0.52	0.30-0.88
Boot dip changed \geq weekly	-0.864	0.330	0.011	0.42	0.22-0.81
Boot dips after house disinfection	-0.755	0.330	0.011	0.47	0.25-0.90
Boot dips after litter/chick arrival	0.456	0.335	}	1.58	0.82-3.04
Dead birds removed from site	-0.445	0.244	0.071	0.64	0.40-1.03

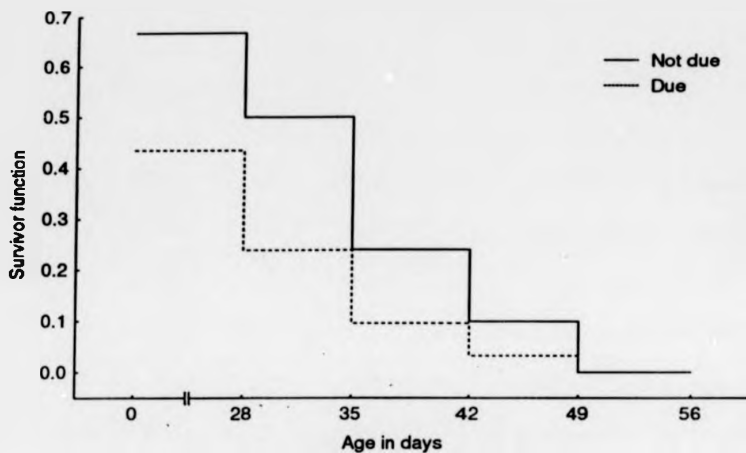


Figure 7.4a Survivor function

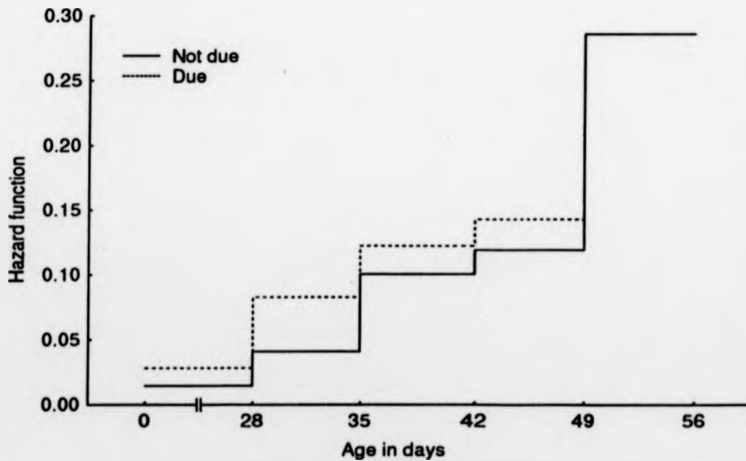


Figure 7.4b Hazard function

Figure 7.4. Effect of the state of repair of the poultry buildings

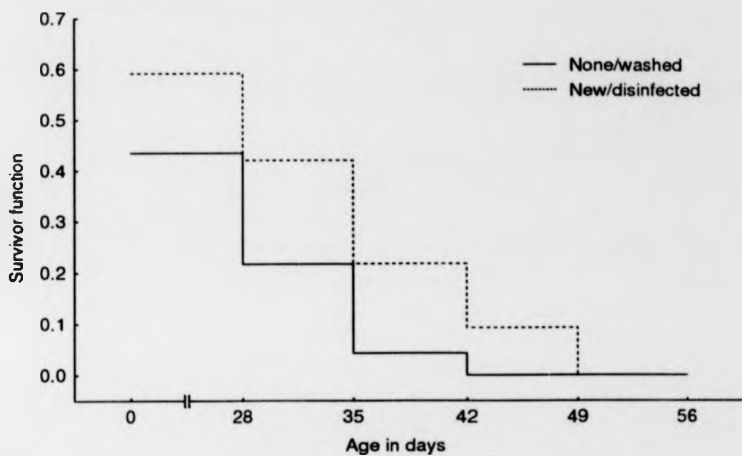


Figure 7.5a Survivor function

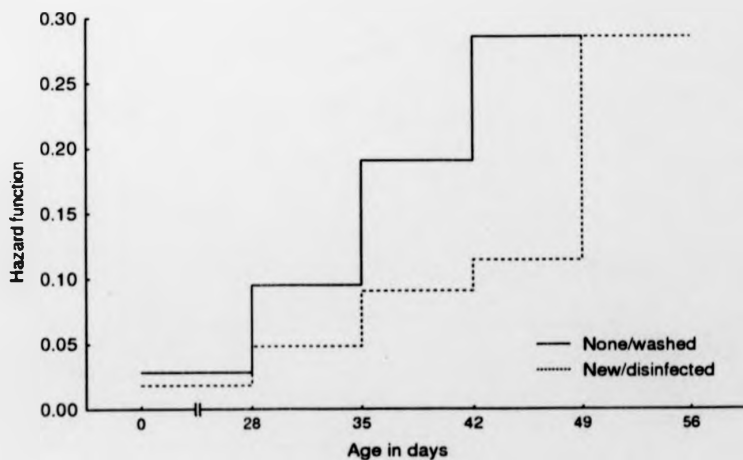


Figure 7.5b Hazard function

Figure 7.5. Effect of the method of cleaning the water header tank

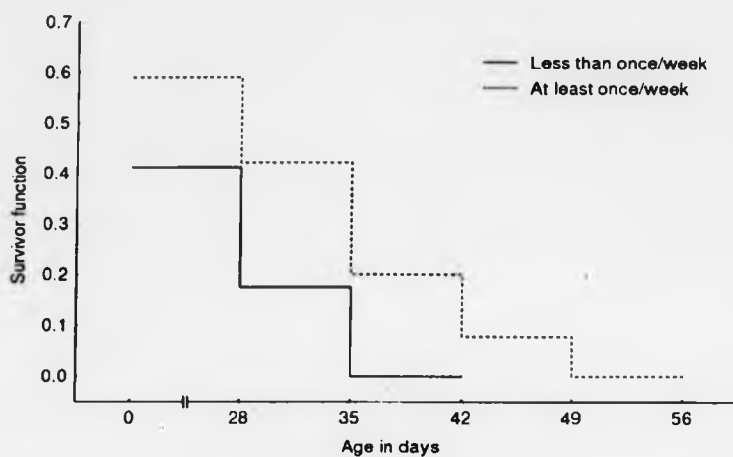


Figure 7.6a Survivor function

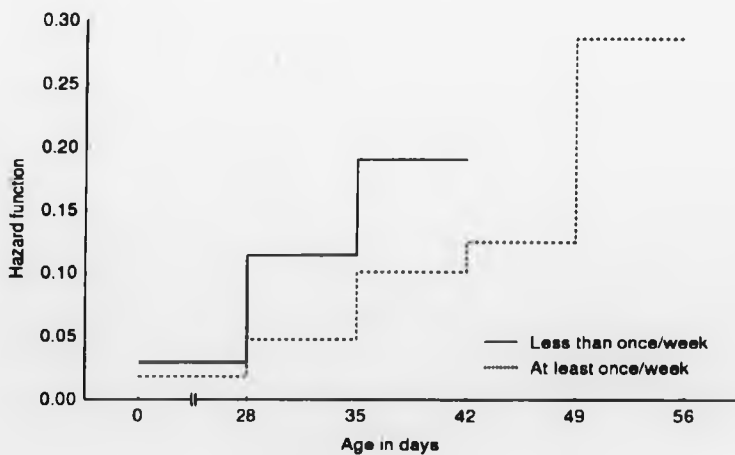


Figure 7.6b Hazard function

Figure 7.6. Effect of the frequency of replenishment of the disinfectant boot dip

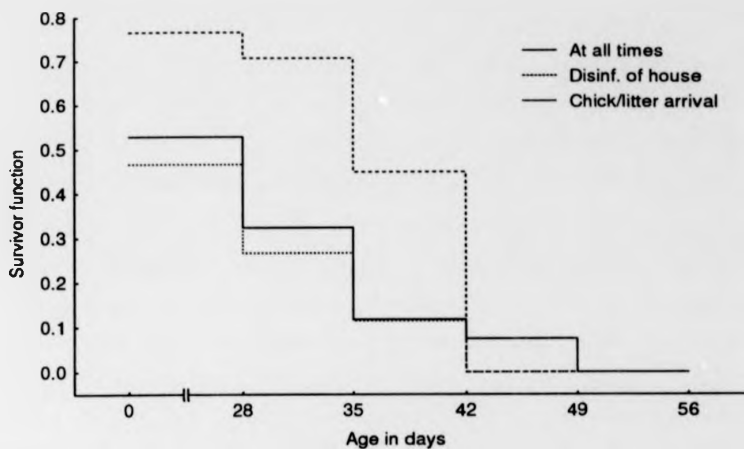


Figure 7.7a Survivor function



Figure 7.7b Hazard function

Figure 7.7. Effect of the disinfectant boot dip procedure

Discussion

This study found a very high incidence of campylobacter infection in British broiler flocks and showed that the risk of infection was strongly associated with chick age. About 40% of flocks were infected by the time the chicks were 3 weeks of age and this approached 100% of flocks by 7 weeks of age. This confirmed previous studies showing a high prevalence of campylobacter infection in British broiler flocks (chapter 6, Humphrey and others 1993). It was also shown that when a flock was infected virtually all cloacal swabs were positive within a week, indicating that campylobacter infection spreads very rapidly amongst housed broiler chicken as shown in a number of other studies (Smitherman and others 1984, Engvall and others 1986, Lindblom and others 1986, Jacobs-Reitsma and others 1995a).

However, some flocks remained free of infection until slaughter and the survival analysis indicated that a number of management factors acted as predictors of the age at which broiler flocks were infected with campylobacter. Therefore, it may be possible to use on-farm intervention measures to extend survival time and reduce the prevalence of campylobacter infection at slaughter. The study emphasised the greater risk of campylobacter colonisation of birds slaughtered at 6-7 weeks of age compared to birds slaughtered at 4-5 weeks of age. It is therefore appropriate to consider risk reduction at the abattoir by, for example, slaughtering young birds early in the day which is likely to result in reduced cross-contamination of carcasses during processing.

A number of approaches were used to model the data but all gave similar results, with minor exceptions. Perhaps the most appropriate model was the discrete time model as the model was able to account for the interval grouped survival times resulting from the scheduled screening times used in the study. If the number of intervals are not too small and the time between successive examinations not too large then it has been shown that the results of a discrete time model will be similar to a PHR model which assumes survival times to be continuous (Efron 1988). All variables, except dead bird disposal, were significant at $p < 0.05$ in the discrete time model.

A number of hypotheses were investigated by the study but it should be remembered that the study had insufficient power to detect factors with only a small effect on risk or factors with an effect on risk but either a very high or very low prevalence in the population. This limitation is mentioned in the discussion, where appropriate.

There was no evidence that rodents were an important source of campylobacter in this population. However, all sites operated a rodent control programme and data relating to the presence of rodents on sites indicated that the control programme was mostly effective. Only 7 sites reported evidence of significant rodent populations and, in a study of this size, this low level of exposure may result in a true effect on risk being undetected. Therefore, rodents cannot be ignored as potential sources of infection as other studies have identified them as carriers of infection (chapter 6, Annan-Prah and Janc 1988, Kapperud and others 1993) but in this population of intensively produced broiler flocks with adequate rodent control operations, rodents were not responsible for the majority of campylobacter infections.

The study investigated whether campylobacter infection persisted in the environment of sites to infect successive flocks. Infection was not predictable by campylobacter status of the previous flock but, unfortunately, the power to detect such an association was poor as most flocks were infected. Molecular typing studies, currently in progress, may be more conclusive. A similar lack of association with infection status of successive flocks was reported by Berndtson and others (1996b). Despite the fact that many cleansing and disinfection errors were apparent, culturable campylobacters were not detected after house disinfection. However, there was a strong association between the state of repair of the broiler house and the age at which infection was detected. Half of all flocks housed in buildings in a good state of repair were free of infection at 35 days of age compared to less than a quarter of flocks housed in buildings in need of repair. This was most likely to reflect either the inability to adequately clean houses which were in poor repair and consequent carry over of infection between flocks or impaired physical barriers between a potentially contaminated external environment and the chicks in these houses, or a combination of both. Both the age of the building and the state of repair of the equipment were

correlated with the state of repair of the poultry houses.

The effective use of boot dips increased the survival time significantly. Disinfectant solutions in boot dips should be replenished at least once a week and certainly if there has been a build up of organic matter or the solution has been diluted in any way. Boot dips should be used at all times after disinfection of the poultry houses and not just after arrival of the litter or chicks. The association with boot dip procedure may be a reflection of general standards of site hygiene but may more specifically indicate that farm workers' footwear was responsible for introducing infection to chicks. Other studies have similarly concluded that farm workers are important in transmitting campylobacter to broiler flocks (Humphrey and others 1993, Berndtson and others 1996a, Berndtson and others 1996b, van de Giessen and others 1996).

The analyses indicated that disinfection of the water header tank had a protective effect and this appeared to be more important than the type of drinker system used. More than 80% of flocks in the study used a municipal chlorinated water supply, which was unlikely to be a primary source of campylobacter. However, the water system would be a very potent means of rapidly spreading infection to chicks and it seems pertinent to ensure adequate sanitization of equipment and water to minimise the risk of campylobacter contamination of the drinking system. Other studies have highlighted the risk of infection from un-disinfected drinking water (Kapperud and others 1993, Pearson and others 1993) and private supplies should be adequately treated before use.

There was evidence that chain feeding equipment was associated with an increased hazard of infection. Again, this emphasises the necessity to clean and disinfect such equipment thoroughly. On-site disposal of dead birds also increased the risk of infection and this may be via environmental contamination. It is advisable that all dead birds are removed promptly from the broiler house and site and handled as potentially contaminated with campylobacter.

Flock size was related to risk of infection as it was shown that survival time was

reduced as site size increased. However, survival was not associated with parent broiler company or with the season of the year. The lack of a seasonal association was rather surprising as studies in other countries have reported that infection is more common in the summer and autumn than the winter or spring (Annan-Prah and Janc 1988, Jacobs-Reitsma and others 1994, Stern 1995). This may reflect differing climatic conditions in Great Britain or the propensity of controlled environment broiler housing. Another British study also failed to find seasonal differences in campylobacter incidence (Humphrey and others 1993).

Visits to the site by abattoir personnel and equipment for the collection of birds for slaughter did not have a significant effect on the risk of infection to remaining birds on the site. This showed that the suggestion of risk seen in other studies (chapter 5, Jacobs-Reitsma and others 1994, Berndtson and others 1996a) may be explained by the confounding effect of the age of the birds. However, the power to detect an association between infection and visits by abattoir personnel was relatively poor in this study as nearly three quarters of the flocks were infected prior to any birds being slaughtered so only the remainder were considered to be at risk from this exposure. Therefore, this potential source of campylobacter infection cannot be disproved with certainty by the study but could not, in any case, account for the majority of flock infections. It is known that flocks can be colonised with multiple strains and it has been noted in other studies that the serotype distribution changed during the production cycle (Jacobs-Reitsma and others 1995b). Therefore, the molecular typing studies may provide a further insight as the distribution of strains in flocks will be examined in relation to this exposure.

The risk of flock infection was shown to increase with age but the effects of the modelled predictors of survival were constant over time and none of the measured time varying exposures were explanatory. Thus, some other undefined factors which are integral to age effect the risk of campylobacter infection in broiler chicks. Some experimental studies suggest that broiler chicks are equally susceptible to campylobacter infection throughout their lifespan (Stern 1988, Shanker and others 1990, Kazwala and others 1992) but Kaino and others (1988) found that the minimum

infective dose depended on the campylobacter strain used and the age of the chicks. Maternal antibodies have been detected in newly hatched chicks which disappear by 2 weeks of age (Myszewski and Stern 1990, Cawthraw and others 1994) and it remains possible that passive immunity may confer protection upon young chicks in the field situation. Alternatively environmentally stressed organisms may be poor colonisers requiring *in vivo* passage to enhance colonisation potential (Cawthraw and others 1996). Thus an increased risk with age may be associated with increasing colonisation potential of environmental campylobacters. It has also been hypothesised that the caecal microflora may be inhibitory to campylobacters at early ages (Humphrey and others 1993).

In conclusion, it appeared from this study that campylobacter infection of intensively produced broiler chicken in Great Britain is widespread. Infection was strongly dependent on the age of the birds and this association should be further investigated as understanding the mechanisms responsible for variation in chick susceptibility in the field may lead to the development of control measures such as competitive exclusion or vaccination. In addition to the age effect, a number of management factors were shown to have an independent effect on the risk of infection. These were generally similar to those found in the previous cross-sectional survey (chapter 6) and as flocks examined in the cross-sectional survey were not involved in the present study the hypotheses put forward by the former have been appropriately tested here. The most important predictors of infection were related to effective hygiene barriers such as housing birds in buildings in a good state of repair, appropriate use of disinfectant boot dips outside poultry houses and a high standard of cleansing and disinfection of drinking water supply equipment. It seems most likely that infection was introduced sporadically to chicks from the external site environment, perhaps by poultry farm workers or wildlife vectors. Therefore, interventions aimed at improving hygiene barriers on broiler sites are most likely to reduce the prevalence of campylobacter infection at slaughter. Such measures may be relatively inexpensive compared with modification of the slaughter process. The effectiveness of strict hygiene procedures have been described as being successful in small studies by van de Giessen and others (1992), Humphrey and others (1993), Jacobs-Reitsma and

others (1995a) and Berndtson and others (1996b) but have not been evaluated in a controlled trial. To this end, a large scale comparative trial to investigate the effectiveness of on-farm hygiene interventions to prevent campylobacter infection of broiler chicken has been initiated and results are expected later this year (1997).

Acknowledgements

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CHAPTER 8

General Discussion

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Introduction

Salmonella and campylobacter bacteria are recognised as the leading causes of infectious diarrhoeal disease in man in Great Britain and many other developed countries. The incidence of these diseases has increased dramatically in recent years. This has been partially attributed to increased awareness and surveillance but the data support a true rise in incidence, particularly during the last decade. A number of factors may have contributed to the rise in food-borne illnesses but many, such as the intensification of farming systems and changes in food eating habits, are largely unavoidable consequences of the continuous development of industrialised nations. We are now faced with the effects of a susceptible population consuming foods which suffer from widespread contamination by micro-organisms. The problem of controlling these diseases must be addressed with some urgency as they are responsible for an increasing burden on the economy. There is currently a high level of public awareness of food safety issues which has been the direct result of recent food scares in Great Britain such as salmonella in eggs and both Bovine Spongiform Encephalopathy and *E. coli* 0157 in beef. Consequentially, there is increasing political pressure to provide the general public with assurances on food safety.

The salmonella in eggs food scare occurred as a result of an unfortunate remark made in 1988 by the then Junior Health Minister regarding the level of salmonella contamination of the egg laying sector of the poultry industry. Widespread media attention caused a high level of public anxiety and consequently a large drop in egg sales. Government measures were rapidly introduced in an attempt to control salmonella in laying hens and breeding birds to allay public concern. However, despite an apparent marked reduction in incidence of *S. enteritidis* infection in poultry breeding flocks, there has been only a minor reduction in the level of human disease. The possible reasons for this anomaly are discussed later. The egg industry was severely effected by the food scare and egg consumption remains more than 40% below the 1985 level (MAFF 1996). The control policy has also been a considerable cost to the Government. This has highlighted the vulnerability of the agricultural sector to the effects of such food scares and, although the risks to public health are paramount and should not be trivialised, the added sensitivity of the consumer to

these issues can lead to a public response which is out of proportion to the health risk attached. The high profile nature of these diseases has benefited researchers as funding levels have increased but at the same time posed problems in obtaining co-operation within the farming community, particularly for field based epidemiological projects. Care is needed on publication of the results of studies to ensure that findings are not misinterpreted or become the subject of media hyperbole.

Poultry meat is recognised as a major source of both salmonella and campylobacter and table eggs are also a source of the former. As a consequence, in recent years, there has been a great deal of research conducted to address the problem of controlling these diseases within the poultry industry. It is important to remember that both these bacteria are usually carried asymptotically by apparently normal, healthy birds. There are a number of points at which control measures can be directed to decrease the risk of human infection and these include measures to reduce the level of infection in the live bird, decontamination methods at the processing plant and food hygiene education at all levels of the food chain. Current hygienic poultry processing methods are able to produce carcasses which are not contaminated by enteropathogens providing the live birds arriving for slaughter are free from infection. However, even a low prevalence of salmonella or campylobacter infection in the birds can result in widespread carcass contamination and slaughter house interventions have so far had limited success in reducing this cross-contamination. Therefore, much attention is currently directed at controlling infection in the live bird. The studies described in this thesis have contributed to our understanding of the epidemiology of these infections in poultry and results can now be used to assist in the formulation of effective control strategies. However, it is expected that the ultimate control of these infections will also require control measures to be introduced in the processing plant and the adoption of stricter food hygiene standards by both retailers and consumers.

The primary aims of the studies that form this thesis were to elucidate the epidemiology of salmonella and campylobacter infection in poultry at a national level in order to assist with the development of effective preventive measures to reduce the prevalence of infection with these organisms in the live bird.

The epidemiology of *Salmonella enteritidis* infection of poultry

There are many potential routes by which poultry flocks can become infected with *S. enteritidis*. This is because the organism has a wide host range and can survive in the environment and feedstuffs. In addition, this particular salmonella serotype has the ability to be vertically transmitted from infected parent birds to their progeny via the egg. The epidemiological picture is further complicated as the relative importance of the various sources of infection may be dependent on the bacterial strain involved, the type of poultry enterprise and the stage of the epidemic.

During the late 1980's, *S. enteritidis* PT4 spread rapidly throughout the British poultry industry. The most likely origins of the epidemic were the establishment of infection in primary breeding flocks and the subsequent transmission of infection via the progeny throughout the breeding and production chains and/or widespread feed contamination, although the latter is possibly less plausible. Both are potentially potent routes of rapidly transmitting infection throughout a highly integrated poultry industry, such as that of Great Britain. The origin of the epidemic may never become established with any degree of certainty but it soon became apparent that, despite the introduction of a test and slaughter policy in 1989, the problem persisted in most sectors of the industry with the exception of the primary breeding flocks. By 1990, infection was widespread and it was likely that other factors, such as environmental contamination, were responsible for maintaining the epidemic.

As the limited success of the control policy became evident, the need for an analytical epidemiological study arose. The national case control study aimed to identify the major routes of *S. enteritidis* infection within the parent breeding sector as the eradication of infection in these flocks was of primary concern to ensure the supply of salmonella free chicks to broiler and egg production flocks.

The case control study identified both the feed and the environment as major sources of *S. enteritidis* PT4 infection of parent breeding flocks at this time and the results are summarised in figure 8.1. The farm environment was shown to be an important source of infection as three separate factors which may allow horizontal spread of *S.*

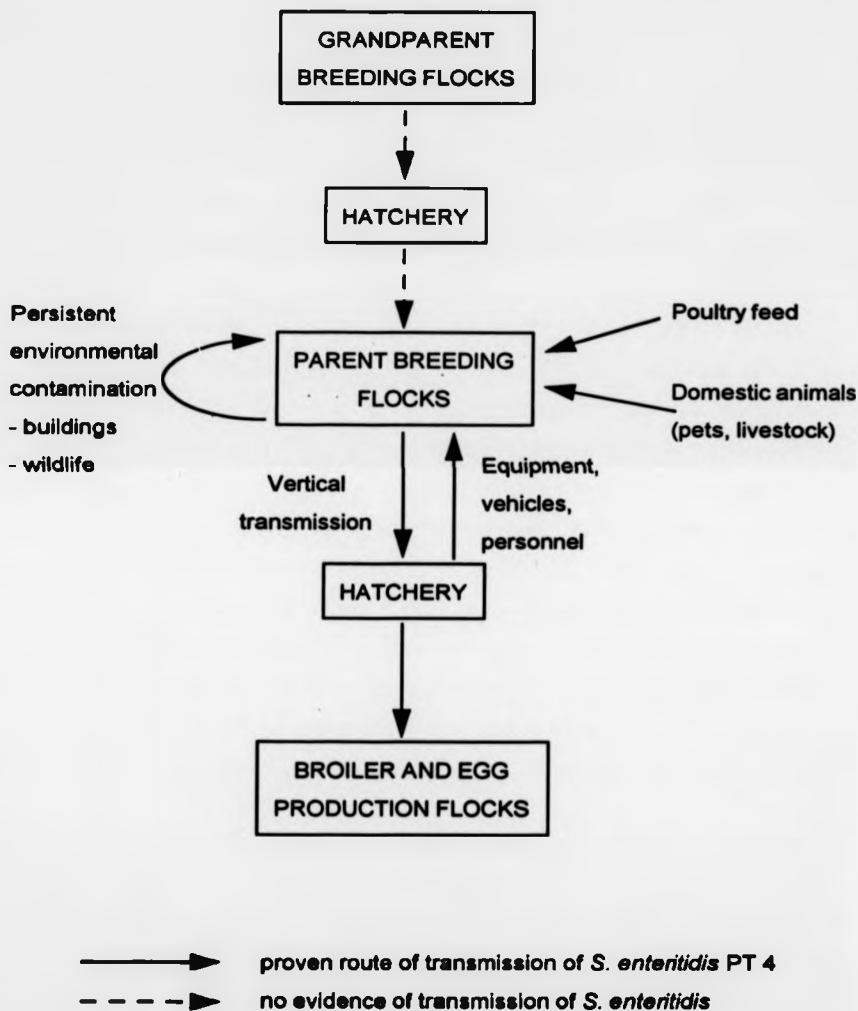


Figure 8.1. Routes of transmission of *S. enteritidis* infection in British poultry parent breeding flocks, based on the results of the national case control study (chapter 3)

enteritidis were found to be significantly associated with the risk of infection. The most important of these was the increased risk of infection associated with a history of salmonella at the poultry site and this indicated that the organism was surviving in the environment including animal reservoirs around the site to infect subsequent flocks of birds. The study showed an association between the incidence of *S. enteritidis* infection at the egg-destination hatchery and the risk of infection in the parent breeding flock. This may have signified that cross-contamination within the hatchery constituted a risk to parent breeding flocks through the use of inadequately disinfected trays and trolleys that were returned to breeder sites or through hatchery vehicles and personnel. The presence of other domestic animals, including cats and dogs, on the site increased the risk of infection which showed that these animals were carriers of infection. Poultry feed was associated indirectly with infection through the protective effect of heat treatment of poultry feed. Although MAFF monitoring reveals only low level salmonella contamination of poultry feed in this country, it is recognised that the monitoring protocols are relatively insensitive because of the problems of sampling large loads. However, there was no evidence from this study of vertical transmission of infection from grandparent flocks. These findings were in general agreement with the only other similar published study which was a retrospective case control study of *S. enteritidis* infection of broiler breeding flocks in the Netherlands (Fris and van den Bos 1995), although the Dutch study failed to associate infection with poultry feed.

The results of the national case control study, although not unexpected, have highlighted the most important areas for control. The voluntary Code of Practice for the control of salmonella in breeding flocks (MAFF 1993), amongst other recommendations, emphasised the importance of the elimination of persistent infection on poultry sites by thorough cleansing and disinfection and vermin control and advocated heat treatment of feed to ensure the delivery of salmonella free poultry feed. Stricter standards of hygiene were adopted at breeding sites as the result of the epidemic and 65% of the control flocks in the national study reported improvements in disease security during the study period (1992-1994). Heat treatment of breeder feed has become more widespread and methods for the effective cleansing and

disinfection of poultry buildings have been developed.

It is likely that these measures, in combination with the slaughter policy and the recent introduction of an effective vaccine, have been responsible for the decline in reports of infection in breeding flocks. The "top down" approach to the control of salmonella in poultry in Great Britain may now have reached the level of the production flocks. Once infection has been eliminated from the breeding flocks, the delivery of salmonella free chicks can be assured and interventions aimed at limiting horizontal transmission of salmonella in production flocks have a chance of success.

The case control study period coincided with the commencement of a decline in number of salmonella incidents reported in the breeding sector. Ten per cent fewer incidents were reported during the first year of the study than during the peak years (1990/1991) and by the end of the study period the number of reports had more than halved. This trend has continued and, in 1996, the number of reported *S. enteritidis* incidents in breeding flocks were only 10% of those at the peak of the epidemic.

There is evidence from the Swedish salmonella control programme that it is possible to reduce levels of salmonella contamination of poultry products to negligible levels through the adoption of stringent control measures. *S. enteritidis* has not been isolated from broilers in Sweden from 1972 and since 1987 only 5 layer flocks have been infected with this serotype. There is a correspondingly low level of domestically acquired salmonella infection in humans. Sweden has operated a salmonella control programme since 1961 and the Swedish poultry industry has not been involved in the *S. enteritidis* pandemic. The control measures were tightened in the light of the pandemic and in essence the control programme currently involves the compulsory quarantine and testing of all imported groups of grandparent birds, voluntary testing of parent flocks during rearing, laying and at hatcheries, and compulsory pre-slaughter testing of broilers and voluntary testing of cull hens. In addition, imported and domestic produced protein and feed mills are continuously monitored for salmonella and the use of heat treated poultry feed has become widespread. This comprehensive strategy incurs a considerable cost which is met by

the producer through insurance. This cost was estimated to be \$0.15 U.S (£0.10 sterling) per kilo chicken produced in 1990 (Wierup and others 1995). Despite the small size of the Swedish industry (50 million broiler chickens are produced annually in Sweden compared with 700 million in Great Britain) and the cost implication, it seems reasonable that other countries, including Great Britain, should aspire to such a high level of salmonella control.

In Sweden, the incidence of *S. enteritidis* infection in both poultry and man is low. However, despite the apparent success of controlling infection in the poultry breeding sector in Great Britain there has been little evidence of a corresponding decrease in human infection. This may reflect the maintenance of infection through horizontal routes of transmission in production flocks, as indicated by recent retail surveys which show poultry meat remains frequently contaminated with salmonella (HMSO 1996), and the lack of effective controls at other points in the food chain (processing plant interventions and consumer food safety education). Control efforts should now be directed at reducing the incidence of infection in broiler and egg production flocks and it is widely agreed that a Hazard Analysis Critical Control Point (HACCP) approach to salmonella control should be adopted within the food chain from producer to consumer (HMSO 1996). The HACCP system involves seven basic principals:-

- 1 identification of hazards, their severity and risk of occurrence;
- 2 determination of critical control points at which hazards can be controlled;
- 3 establishment of critical control point tolerances;
- 4 development and use of monitoring procedures at critical control points;
- 5 identification of actions required in the event of a breakdown in control;
- 6 verification of controls to ensure the HACCP system is working;
- 7 record keeping.

The *S. enteritidis* epidemic has emphasised the insecurity of the British poultry industry to major disease outbreaks. The integrated structure of the industry facilitates the rapid spread of diseases, which can be transmitted through the progeny, and the use of centralised feed supplies carries the danger of widespread infection if contamination of feedstuffs with micro-organisms, such as salmonella, occurs.

Attention should be directed at reducing the risk of the introduction of diseases to production flocks, particularly via breeding flocks and feedstuffs, by increasing the standards of disease security throughout the industry. Again, a HACCP approach to the maintenance of effective biosecurity at poultry sites and feed mills is advocated.

The epidemiology of *Campylobacter jejuni* infection of poultry

The status of our current knowledge of the epidemiology of *C. jejuni* infection in poultry flocks is inferior to that of salmonella infection. This can, in part, be attributed to the relatively recent recognition of the role of thermophilic campylobacters as a cause of human diarrhoeal disease and the requirement for specialised microbiological methods for the isolation of the organism. However, it is now established that campylobacter causes more human illness than salmonella and poultry meat is the main vehicle of infection. Like salmonella, the organism can survive normal hygienic poultry processing and, although *C. jejuni* does not replicate in foods stored under normal conditions, the low infective dose and the high level of surface contamination of chicken carcasses has resulted in widespread human disease.

The origins of infection and mode of transmission of *C. jejuni* within commercial poultry flocks are uncertain. The lack of routine methods of subtyping the organism has also hindered progress. *C. jejuni* is unlikely to be transmitted via the egg. In direct contrast to *S. enteritidis*, egg-associated human illness has not been reported and vertical transmission of infection to poultry flocks is not a recognised route of infection. Therefore, research efforts have been directed at establishing the epidemiology of infection in commercial broiler flocks in order to develop effective control measures. The provision of campylobacter free birds for slaughter is the ultimate aim in the absence of effective carcass decontamination methods.

The collection of studies reported in this thesis have provided a great deal of descriptive epidemiological data as well as highlighting the most important risk factors for *C. jejuni* infection of commercial broiler flocks. This is the first body of work of its kind in Great Britain and the results can be used to formulate campylobacter control strategies.

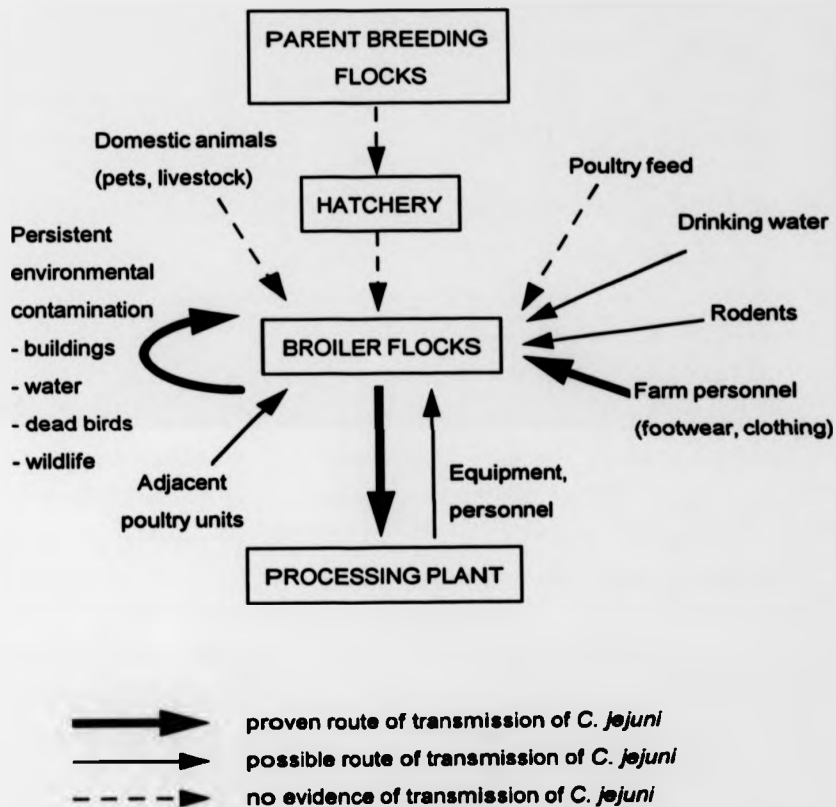


Figure 8.2. Routes of transmission of *C. jejuni* infection in British broiler flocks, based on the results of the epidemiological studies in this thesis (chapters 5-7)

Sampling methods were developed for the efficient detection of campylobacter infection in broiler flocks and two commonly used subtyping methods (Penner serotyping and PCR/RFLP typing) were compared. Cloacal swabs were shown to be a sensitive method for the detection of *C. jejuni* infection and it was apparent that, due to frequent high prevalence flock infection, campylobacter could be detected in broiler flocks with a high degree of certainty by sampling relatively few birds.

The main species of campylobacter carried by poultry was *C. jejuni* which was isolated from more than 95% of infected flocks. *C. jejuni* appeared to be a commensal organism of the avian gut. It was not associated with clinical disease in the poultry flocks studied and there was no evidence of adverse effects on production. The studies established that *C. jejuni* infection was widespread within the British broiler industry. Despite a strong seasonality of human infection, there was no evidence of seasonal variation in prevalence of *C. jejuni* infection in broiler flocks. The national survey reported that at 5-6 weeks of age 45% of flocks were colonised with campylobacter (95% CL: 36.9-53.1) and the longitudinal study showed that this increased to more than 90% of flocks by 7 weeks of age. Infection was strongly related to age but the reasons for the delay in colonisation remain unclear. Once infected, the majority of birds within a flock were rapidly colonised and remained carriers of *C. jejuni* through to slaughter. This contrasts with *S. enteritidis* infection in breeding flocks where low prevalence flock infection was common. However, directly comparable data are not available for broiler flocks.

The studies have identified major routes of campylobacter infection for broiler flocks and these are summarised in figure 8.2. Unlike *S. enteritidis*, there was no evidence of vertical transmission of *C. jejuni* from infected parent flocks or of transmission in poultry feed. The environment appeared to be the main reservoir of infection for broiler flocks. The studies investigated whether campylobacter persisted in the environment of poultry buildings to infect successive flocks. There was a strong association between the state of repair of the poultry buildings and the risk of infection which was thought to reflect either the inability to adequately clean buildings in a poor state of repair or the impaired physical barriers to a potentially

contaminated external environment, or a combination of both. It was also shown that methods of cleansing and disinfection of the poultry buildings after flock depopulation influenced the risk of infection in the subsequent flock. However, despite the fact that many cleansing and disinfection errors were apparent, culturable campylobacters were not detected after house disinfection. The longitudinal study attempted to ascertain whether infection was predictable by the campylobacter status of the preceding flock but was, unfortunately, unable to establish this fact as the power to detect the association was poor as most flocks were infected. The use of hygiene barriers at the entrances to the broiler houses, particularly disinfectant boot dips, significantly reduced the risk of infection indicating that contaminated footwear and possibly clothing worn by farm workers were important sources of campylobacter infection. Drinking water was also shown to be a source of infection although it was unclear as to whether water was a primary source or simply a reservoir for environmental survival of the organism.

Rodents were shown to be possible vectors of infection in the national survey but the longitudinal study indicated that, in the absence of significant rodent populations, *C. jejuni* infection remained common. It was also hypothesised that visits to the site by abattoir vehicles and personnel for the collection of birds for slaughter increased the risk of infection to remaining birds on the site. However, this risk factor remains unproven as it was not possible to correct for the confounding effect of the age of the birds as so few flocks were uninfected by the time of slaughter.

It seems most likely that *C. jejuni* infection is introduced sporadically to chicks from the external environment, perhaps by poultry farm workers or wildlife vectors. Therefore, interventions aimed at improving hygiene barriers on broiler sites are most likely to reduce the prevalence of campylobacter infection at slaughter.

The epidemiology of *S. enteritidis* and *C. jejuni* infections in poultry flocks have been shown to have some similarities but also marked differences. Accordingly, it is likely that effective control measures will differ. This is exemplified by the apparent successful control of salmonella in Swedish broiler flocks (Weirup and others 1995)

whilst campylobacter infection remains common (Berndtson and others 1996a). It is likely that specific on-farm interventions will be required to significantly reduce the prevalence of *C. jejuni* infection in broiler flocks but, as with salmonella, the ultimate reduction of human disease will require control efforts at all points of the food chain. The situation in Sweden may indicate the limited ability of management improvements to control *C. jejuni* infection in the live bird and emphasises the importance of investigating other methods of control such as vaccination, competitive exclusion and processing plant interventions.

Conclusion

The studies have demonstrated the strength of properly designed analytical methods to investigate the epidemiology of zoonotic diseases in livestock enterprises. However, in contrast to studies of human populations, account must be taken of the effect of clustering ("herd effect") in the design or analysis of such studies and methods must be established to correctly identify the infection status of animals as asymptomatic infection may be common or even the norm. These studies are a powerful method for the identification of major routes of infection and results can have a direct influence on the formulation of control strategies. However, the inherent limitations of the studies should be remembered, particularly the potential for the introduction of bias in case control studies. Both salmonella and campylobacter have a complex multi-factorial epidemiology and interactions between risk factors are common. Studies must be of sufficient size to detect the small relative risks that may be associated with the many potential sources of infection and account for the uniformity of management factors in intensive livestock production systems. This is particularly difficult given the relatively small number of individual units associated with a highly developed agricultural sector such as the poultry industry. The uniform management of farms within an individual poultry company can limit the usefulness of epidemiological investigations which are restricted to a single company. The studies reported in this thesis have provided results that can be generalised to the national population as data collection was at a national level and involved most of the major poultry companies operating in Great Britain. There was an extremely good level of co-operation in these studies by the poultry industry despite the sensitivity of

the research. This was achieved by developing close working relationships with individual poultry companies and approaching the British Poultry Meat Federation in the planning stages of the projects to gain the support of their members. Early feedback of the results was provided to the industry in return for this support. The backing of the industry was of paramount importance to the success of the studies.

Major risk factors for *S. enteritidis* and *C. jejuni* infection of commercial poultry flocks have been identified. This knowledge will assist in the development of suitable methods to control these important food-borne zoonotic infections. The studies examined in detail one critical control point, that of infection in the live bird. However, in order to have the maximum impact on human disease, control efforts must be directed at all critical points of the food chain, from the producer to the consumer, and a good deal of further research may be required before a successful HACCP system of control of food-borne infections is established. It is hoped that the present work has made a substantial contribution to the ultimate aim of eliminating poultry as a significant source of human food-borne disease pathogens.

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APPENDICES

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APPENDIX A

A case control study of *Salmonella enteritidis* infection in British poultry breeding flocks

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APPENDIX A.1

The Zoonoses Order 1989

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STATUTORY INSTRUMENTS

1989 No. 285

ANIMALS
ANIMAL HEALTH

The Zoonoses Order 1989

Made - - - - - 28th February 1989*Coming into force* 1st March 1989

The Minister of Agriculture, Fisheries and Food, the Secretary of State for Scotland and the Secretary of State for Wales, acting jointly, in exercise of the powers conferred on them by sections 1, 7(1) and (2), 8(1), 17(1), 23, 29, 72, 86(1), 87(2) and (5)(a) of the Animal Health Act 1981(a) and of all other powers enabling them in that behalf, hereby make the following Order:

Title and commencement

1. This Order may be cited as the Zoonoses Order 1989 and shall come into force on 1st March 1989.

Extension of definitions of "animals" and "poultry"

2. For the purposes of the Act in its application to the presence in animals or poultry of designated organisms -

- (a) the definition of "animals" in section 87(1) of the Act is hereby extended so as to comprise -
 - (i) any kind of mammal except man, and
 - (ii) any kind of four-footed beast which is not a mammal; and
- (b) the definition of "poultry" in section 87(4) of the Act is hereby extended so as to comprise birds of every species.

Interpretation

3. In this Order, unless the context otherwise requires -

"the Act" means the Animal Health Act 1981;

"the appropriate Minister" means, in relation to England, the Minister and in relation to Scotland or to Wales, the Secretary of State;

"approved disinfectant" means a disinfectant for the time being listed in the Diseases of Animals (Approved Disinfectants) Order 1978(b) as approved for use under a general order;

"animal" means any kind of mammal, except man, and any kind of four-footed beast which is not a mammal;

(a) 1981 c. 22; as applied by S.I. 1975/1030, section 86(1) contains a definition of "the Ministers" relevant to the exercise of the statutory powers under which this Order is made. (b) S.I. 1978/32, relevant amending instrument to S.I. 1989/144. (c) S.I. 1975/1030.

"carcase" means the carcase of an animal or of any poultry and includes part of a carcase or any portion thereof;

"designated organism" means an organism designated by article 4 of this Order for the purposes of section 29 of the Act;

"feedingstuff" means feedingstuff whatever its derivation and includes any ingredient used in the preparation of a feedingstuff;

"infected place" means premises declared to be an infected place by a notice served under article 6 of this Order;

"inspector of the Minister" means a person appointed by the Minister to be an inspector for the purposes of the Act and includes a veterinary inspector;

"the Minister" means the Minister of Agriculture, Fisheries and Food and "the Ministers" means the Minister, the Secretary of State for Scotland and the Secretary of State for Wales, acting jointly;

"premises" includes land;

"product" means milk, eggs, wool, meat, offal, dung or other substance directly derived from an animal or from any poultry, whether mixed with any other substance or not, and includes used bedding litter;

"poultry" means birds of any species;

"veterinary inspector" means a veterinary inspector appointed by the Minister.

Designation of organisms for the purposes of section 29

4.—(1) The following organisms, being organisms which, when carried in animals or poultry, constitute in the opinion of the Ministers a risk to human health, are hereby designated for the purposes of section 29 of the Act, that is to say —

- (a) organisms of the genus salmonella; and
- (b) organisms of the genus brucella.

(2) The provisions of the Act listed in Schedule 1 to this Order shall apply in relation to the presence of a designated organism in an animal or in any poultry as if the presence of the organism were a disease to which the Act applies.

Inspections, taking samples etc.

5.—(1) An inspector of the Minister who enters any land, building or other place in exercise of his powers under section 63(9) of the Act or who enters any pen, shed, land or other place in exercise of his powers under section 64(1) of the Act may —

- (a) carry out such inquiries, examinations and tests; and
- (b) take such number of birds and such other samples,

as are necessary to ascertain whether any designated organism exists or has existed there.

(2) An inspector of the Minister may, for the purposes of identification, mark any animal, poultry, carcase, product or feedingstuff or other thing in relation to which any of the powers under paragraph (1) above has been exercised.

Infected places

6.—(1) Where a veterinary inspector has reasonable grounds for supposing that there is or has been on any premises an animal or any poultry or feedingstuff in which a designated organism is or was present, or the carcase of such an animal or poultry or a product derived from such an animal or poultry, he may serve a notice on the occupier of the premises declaring them to be an infected place.

(2) A veterinary inspector may, by the same notice as is referred to in paragraph (1) above or by a further notice served in the like manner, -

- (a) prohibit the movement of any animal, poultry, carcase, product or feedingstuff into or out of the infected place except under the authority of a licence issued by a veterinary inspector and in accordance with any conditions subject to which the licence is issued;
- (b) prohibit the movement out of the infected place of any dung, droppings, equipment, utensil, appliance, vehicle or other thing except under the authority of a licence issued by a veterinary inspector and in accordance with any conditions subject to which the licence is issued;
- (c) require any animal, poultry, carcase, product or feedingstuff specified in the notice to be detained in such part of the infected place as may be so specified, except that any such animal, poultry, carcase or product or feedingstuff may be moved out of the infected place under the authority of a licence issued by a veterinary inspector and in accordance with any conditions subject to which the licence is issued;
- (d) require any animal, poultry, carcase, product or feedingstuff specified in the notice to be isolated from any other animal, poultry, carcase, product or feedingstuff or from human beings (other than those persons whose presence is necessary for the purposes of providing care and attention for them).

(3) A notice served under this article may at any time be revoked or varied by a further notice served by a veterinary inspector on the occupier of the infected place.

(4) Any notice which may be served or licence which may be issued by a veterinary inspector under this article may be served or issued by an inspector of the Minister acting under the direction of a veterinary inspector.

Cleansing and disinfection

7.—(1) An inspector of the Minister may, by notice in writing served on the occupier of an infected place or of any other premises in which a designated organism is known or suspected to have been present, require him to cleanse and disinfect at his own expense or, if the notice so specifies, at the expense of the appropriate Minister, with an approved disinfectant and in such manner and within such period as may be specified in the notice -

- (a) all or any part of the infected place or other premises; and,
- (b) any equipment, utensil, appliance or other thing used there in connection with any animal, poultry, carcase, product or feedingstuff.

(2) An inspector of the Minister may, by notice in writing served on the owner or person in charge of any vehicle which is used for the carriage of any animal, poultry, carcase, product or feedingstuff in which a designated organism is known or suspected to have been present, require him to cleanse and disinfect at his own expense or, if the notice so specifies, at the expense of the appropriate Minister, with an approved disinfectant and in such manner and within such period as may be specified in the notice -

- (a) the vehicle; and
- (b) any equipment, utensil, appliance or other thing used in connection with such carriage.

(3) If any person on whom a notice has been served under paragraph (1) or (2) above fails to comply with the requirements of the notice, an officer of the appropriate Minister may, without prejudice to any proceedings arising out of such default, carry out or cause to be carried out the requirements of the notice, and, except where the requirements of the notice are to be carried out at the expense of the appropriate Minister, the amount of any expenses reasonably incurred by him in doing so shall be recoverable as a civil debt by the appropriate Minister from the person in default.

Reporting of presence of designated organisms

8.—(1) Subject to paragraphs (3) and (4) below, where the presence of a designated organism in a sample taken from an animal or bird, or from the carcase, products or surroundings of an animal or bird or from any feedingstuff is identified by a laboratory examination or by a serological or other examination carried out elsewhere than at a laboratory, the person in charge of the laboratory, or, in the case of an examination carried out elsewhere than at a laboratory, the person carrying out such examination shall forthwith make to a veterinary officer of the Minister a written or oral report containing the particulars specified in Schedule 2 to this Order.

(2) A person who is under an obligation to make a report under paragraph (1) above shall, if so required by an officer of the appropriate Minister, supply that officer with a culture of the designated organism in respect of which that obligation arose.

(3) Nothing in paragraph (1) above shall require a person to make a report where his knowledge or suspicion of the presence of a designated organism results from an identification made by or on behalf of the appropriate Minister.

(4) Where a designated organism has been deliberately introduced into an animal or bird in a research establishment and neither the animal or bird, nor any other animal or bird to which the organism might be transmitted, nor any carcase of, or product derived from, any such animal or bird, is to be sold or otherwise disposed of either for human consumption or for consumption by animals or birds or in any other way which may create a risk to human health, the fact that the presence of the organism is identified in a sample taken from the animal or bird shall not give rise to any obligation to make a report under paragraph (1) above.

(5) For the purposes of this article —

- (a) "animal" means a bull, cow, steer, heifer, calf, horse, deer, sheep, goat, pig or rabbit;
- (b) "bird" means a domestic fowl, turkey, goose, duck, guinea-fowl, pheasant, partridge, quail or pigeon;
- (c) "research establishment" means an establishment carrying out research into a designated organism;
- (d) "veterinary officer of the Minister" means an officer of the Minister who is a person registered in the register of veterinary surgeons or in the supplementary veterinary register; and
- (e) each serotype of the genus salmonella shall be regarded as a separate organism and a person shall not be absolved from an obligation under paragraph (1) above to make a report in respect of an animal, bird or feedingstuff or in respect of the carcase, products or surroundings of an animal or bird by reason of the fact that a report in relation to another serotype of the genus salmonella, or to salmonella of an unidentified serotype, has already been made in respect of that animal, bird, carcase or feedingstuff or in respect of those products or surroundings.

Offences

9. Any person who, without lawful authority or excuse, proof of which shall lie on him, —

- (a) defaces, obliterates or removes any mark applied to any animal, poultry or carcase under article 5(2) of this Order;
- (b) contravenes any provision of this Order or any provision of a licence issued or of a notice served under this Order; or
- (c) fails to comply with any such provision or with any condition of such a licence or notice; or
- (d) causes or permits any such contravention or non-compliance, (commits an offence against the Act.)

687.05

Local authority to enforce Order

10. The provisions of this Order shall, except where otherwise expressly provided, be executed and enforced by the local authority.

Revocation

11. The Zoonoses Order 1975(a) is revoked.

In Witness whereof the Official Seal of the Minister of Agriculture, Fisheries and Food is hereunto affixed on 26th February 1989.



John MacGregor
Minister of Agriculture, Fisheries and Food

28th February 1989

Sanderson of Bowden
Minister of State, Scottish Office

28th February 1989

Peter Walker
Secretary of State for Wales
687.06

SCHEDULE 1

687.06
Article 4(2)

PROVISIONS OF THE ANIMAL HEALTH ACT 1981 APPLIED
IN RELATION TO THE DESIGNATED ORGANISMS WITH A VIEW
TO REDUCING THE RISK TO HUMAN HEALTH FROM
THOSE ORGANISMS

<i>Provision</i>	<i>Description</i>
Section 1	General powers of Ministers to make Orders
Section 3	Expenditure for eradication of disease
Section 4	Offences relating to section 3
Section 5	Power to provide veterinary services
Section 7	Cleansing and disinfection
Section 8	Movement generally
Section 10	Importation
Section 11	Export to Member States
Sections 17, 18 and 23	Infected places and areas
Section 25	Movement of diseased or infected animals
Section 27	Exclusion of persons from infected areas and places
Section 28	Seizure of diseased or suspected animals
Section 31(e)	Power to slaughter in relation to poultry
Section 32	Slaughter in other diseases
Section 33	Additional staff and expenses relating to slaughter
Section 34	Slaughter and compensation generally
Section 35	Seizure and disposal of things by means of which disease may be carried or transmitted
Section 36	Compensation for seizure
Section 50	Local authorities for the purposes of the Act
Section 51	Local authorities and their districts
Section 52	Inspectors and other officers of local authorities
Section 53	Borrowing powers of local authorities
Section 59	Default of local authorities
Section 60	Powers and duties of constables
Section 63	General powers of inspectors
Section 64	Powers of inspectors as to poultry
Section 66	Refusal of admission to land etc. and obstruction
Section 67	Issue of false licences etc.
Section 68	Issue of licences etc. in blank
Section 69	Falsely obtaining licences etc.
Section 71	Other offences as to licences
Section 72	Offences made and declared by and under the Act
Section 73	General offences
Section 75	Punishment for offences
Section 76	Certain importation offences triable either summarily or on indictment
Section 77	Money recoverable summarily as a civil debt
Section 78	Right of appeal
Section 79	Evidence and procedure under the Act
Section 80	Yearly returns to Parliament
Section 81	Reports to Ministers
Section 83	Form and service of instruments
Section 84	Fees
Section 86	Ministers and their functions
Section 87	Meaning of "animals" and "poultry"
Section 89	Other interpretation provisions
Section 90	Application to hovercraft
Section 91	Publication etc of Orders
Section 92	General application to Scotland
Schedule 2	Matters about which provision may be made in an Order under section 10
Schedule 3, paragraph 5	Slaughter of poultry

SCHEDULE 2

Article 8(1)

PARTICULARS REQUIRED TO BE GIVEN IN A WRITTEN OR ORAL REPORT OF THE PRESENCE OR SUSPECTED PRESENCE OF A DESIGNATED ORGANISM

1. The known or suspected identity of the organism.
2. The nature of the sample from which the designated organism was isolated.
3. The address of the premises at which the sample was taken and the name of the owner or person in charge of those premises (stating which).
4. The species and type of animal or bird from which the sample was taken (if appropriate).
5. The date on which the sample was examined.
6. The name and address of the person submitting the report.
7. In the case of a written report, the signature of the person submitting the report and the date.

EXPLANATORY NOTE

(This note is not part of the Order)

Section 29 of the Animal Health Act 1981 ("the Act") empowers Ministers, with a view to reducing the risk to human health from any organism carried in animals or poultry, to make an order designating any organism which, in their opinion, constitutes such a risk and to apply any provision of the Act to that organism.

This Order, which revokes and re-enacts, with amendments, the provisions of the Zoonoses Order 1975, designates (as did the 1975 Order) organisms of the genus salmonella and the genus brucella for the purposes of section 29 of the Act (article 4(1)) and applies certain provisions of the Act (including powers relating to the slaughter of poultry) to those organisms with a view to reducing any risk to human health from them (article 4(2) and Schedule 1).

The Order continues to provide for –

- (a) the declaration as an infected place of premises on which there is or has been an animal or any poultry in which a designated organism is or was present and the imposition, by notice, of movement restrictions and isolation requirements (article 6); and
- (b) the cleansing and disinfection of premises and vehicles in which a designated organism is known or suspected to have been present (article 7).

The changes of substance made by this Order are the inclusion of provisions which –

- (a) empower a Ministry inspector who has entered any premises in exercise of his powers under section 63(9) or section 64 of the Act to carry out such inquiries, examinations and tests and to take such samples as are necessary to ascertain whether any designated organisms are or have been present there (article 5); and
- (b) extend the requirement for making a report concerning the identification of a designated organism in a sample taken from certain animals, birds etc. (article 8 and Schedule 2).

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APPENDIX A.2

The Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989

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In
Original

 STATUTORY INSTRUMENTS

1989 No. 1963

ANIMALS

ANIMAL HEALTH

The Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989

Made - - - - - 19th October 1989

Coming into force

Articles 3 and 4

Remainder

2nd April 1990

26th October 1989

The Minister of Agriculture, Fisheries and Food, the Secretary of State for Scotland and the Secretary of State for Wales, acting jointly, in exercise of the powers conferred on them by sections 1, 72 and 86(1) of the Animal Health Act 1981(a) and of all other powers enabling them in that behalf, hereby make the following Order:

Title and commencement

1.—(1) This Order may be cited as the Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989 and, except for articles 3 and 4 shall come into force on 26th October 1989.

(2) Articles 3 and 4 shall come into force on 2nd April 1990.

Interpretation

2.—(1) In this Order, unless the context otherwise requires—

“the Act” means the Animal Health Act 1981;

“the appropriate Minister” means, in relation to England, the Minister, and in relation to Scotland or to Wales, the Secretary of State;

“approved disinfectant” means a disinfectant for the time being listed in the Diseases of Animals (Approved Disinfectants) Order 1978(b) as approved for use under a general order;

“authorised laboratory” means a laboratory authorised in writing by the Minister for the purposes of this Order;

“breeding flock” means any flock of poultry consisting of not less than 25 birds which are kept (or are being reared) for the production of hatching eggs or birds for (in either case) sale or supply for breeding purposes or for the production of eggs or for meat;

“the Breeding Flocks Register” means the register kept by the appropriate Minister under article 3(2);

“chicks” means poultry less than 72 hours old which have not been fed;

“chick box liner” means any material used to line a box or other container in which chicks are transported from a hatchery to any rearing premises.

(a) 1981 c 22, as applied by S.I. 1989/285, section 86(1) contains a definition of “the Ministers” relevant to the exercise of the statutory powers under which this Order is made

(b) S.I. 1978/32, relevant amending instruments are S.I. 1989/144 and 1555

"composite faeces sample" means a sample of faeces consisting of a number of individual samples of faeces calculated in accordance with the appropriate provisions of Part I of Schedule 3 each of which weighs not less than 1 gram and is taken from a site selected at random to represent the house or group of houses on the premises from which it is taken;

"hatching eggs" means eggs intended for incubation;

"the Hatcheries Register" means the register kept by the appropriate Minister under article 4(2);

"hatchery" means any premises, with a total incubator capacity of not less than 1,000 eggs, on which the eggs of poultry are incubated or hatched and from which chicks are sold or supplied;

"house" means—

(a) a building (including a shed); or

(b) a part of a building separated from other parts of that building by a solid partition and having its own ventilation system;

"inspector" means a person appointed to be an inspector for the purposes of the Act by the Minister or by a local authority and, when used in relation to an officer of the Minister, includes a veterinary inspector;

"laboratory" means any laboratory which has the necessary facilities and personnel for carrying out tests on samples mentioned in Parts I and II of Schedule 3 in accordance with the provisions of Part III of that Schedule;

"the Minister" and "the Ministry" mean respectively the Minister and the Ministry of Agriculture, Fisheries and Food,

"poultry" means domestic fowls, turkeys, geese or ducks;

"premises" includes land;

"rearing premises" means any premises on which chicks are placed for rearing as replacement breeding stock;

"sanitised", in relation to any eggs, means—

(a) fumigated with formaldehyde;

(b) sprayed with or immersed in an egg shell disinfectant in accordance with the manufacturer's instructions; or

(c) made hygienic by any other method approved by the Minister;

"vermin" means rats, mice, flies or cockroaches;

"veterinary inspector" means a veterinary inspector appointed by the Minister

(2) Until the coming into force of articles 3 and 4 any reference in this Order to a person whose name is entered in the Breeding Flocks Register or in the Hatcheries Register in respect of any premises shall be a reference to a person who is keeping a breeding flock on any premises or (as the case may be) to a person who is using any premises as a hatchery and whose name will be required to be entered in the Breeding Flocks Register or in the Hatcheries Register in respect of those premises in accordance with article 3 or 4 when those articles come into force.

(3) Any reference in this Order to the supply of any hatching eggs, chicks or birds shall be deemed to include the transfer of them from one premises to any other premises whether or not in the same ownership or occupation for breeding purposes or for the production of eggs or for meat.

(4) After 1st June 1990 any reference in this Order to a laboratory shall be a reference to an authorised laboratory.

(5) Any reference in this Order to a numbered article or Schedule is a reference to the article or Schedule bearing that number in this Order.

Registration of breeding flocks

3.—(1) Subject to paragraph (9) below, no person shall keep a breeding flock on any premises unless his name is entered in the Breeding Flocks Register in respect of the premises on which the flock is kept.

(2) The appropriate Minister shall keep, for the purpose of paragraph (1) above, a register of persons as being persons entitled to keep a breeding flock on premises in respect of which their names are entered in the register.

(3) Where a person makes an application in writing to the appropriate Minister for his name to be entered in the Breeding Flocks Register in respect of any premises the appropriate Minister shall, subject to paragraphs (4) and (5) below, enter his name in the Breeding Flocks Register in respect of those premises and shall issue to the applicant a certificate of such registration.

(4) The appropriate Minister shall refuse to enter the name of any person in the Breeding Flocks Register in respect of any premises unless all the particulars specified in Part I of Schedule 1 are notified to him in writing.

(5) The appropriate Minister shall refuse to enter the name of any person in the Breeding Flocks Register in respect of any premises if, as a result of an inspection carried out on the premises by an inspector of the Minister, he is satisfied that any of the requirements specified in Part I of Schedule 2, in so far as it relates to the location or to the construction or adaption of premises on which a breeding flock is kept, is not being complied with or will not be able to be complied with by the date on which registration would otherwise have been effected.

(6) The registration of the name of a person in the Breeding Flocks Register in respect of any premises shall remain in force for a period of one year.

(7) A person who wishes to renew the registration of his name in the Breeding Flocks Register in respect of any premises for a further period of one year from the date of its expiry shall make an application in writing to the appropriate Minister before such date.

(8) On receipt of an application for the renewal of the registration of the name of a person in the Breeding Flocks Register in respect of any premises the appropriate Minister shall renew such registration for a further period of one year from the date of its expiry unless, as a result of an inspection carried out on the premises by an inspector of the Minister, he is satisfied that the applicant has failed to comply with any of the requirements specified in Part I of Schedule 2 relating to the keeping of a breeding flock on those premises.

(9) The provisions of paragraph (1) above shall not apply in the case of a breeding flock consisting of birds which are kept solely for the production of hatching eggs or chicks for use (in either case) in the manufacture of vaccines or for research or other scientific purposes.

Registration of hatcheries

4.—(1) Subject to paragraph (9) below, no person shall use any premises as a hatchery unless his name is entered in the Hatcheries Register in respect of those premises.

(2) The appropriate Minister shall keep, for the purpose of paragraph (1) above, a register of persons as being persons entitled to use as a hatchery any premises in respect of which their names are entered in the register.

(3) Where a person makes an application in writing to the appropriate Minister for his name to be entered in the Hatcheries Register in respect of any premises the appropriate Minister shall, subject to paragraphs (4) and (5) below, enter his name in the Hatcheries Register in respect of those premises and shall issue to the applicant a certificate of such registration.

(4) The appropriate Minister shall refuse to enter the name of any person in the Hatcheries Register in respect of any premises unless all particulars specified in Part II of Schedule 1 are notified to him in writing.

(5) The appropriate Minister shall refuse to enter the name of any person in the Hatcheries Register in respect of any premises if, as a result of an inspection carried out on the premises by an inspector of the Minister, he is satisfied that any of the requirements specified in Part II of Schedule 2, in so far as it relates to the location or to the construction or adaption of premises which are used as a hatchery, is not being complied with or will not be complied with by the date on which registration could otherwise have been effected.

(6) The Registration of the name of a person in the Hatcheries Register in respect of any premises shall remain in force for a period of one year.

(7) A person who wishes to renew the registration of his name in the Hatcheries Register in respect of any premises for a further period of one year from the date of its expiry shall make an application in writing to the appropriate Minister before such date.

(8) On receipt of an application for the renewal of the name of a person in the Hatcheries Register in respect of any premises the appropriate Minister shall renew such registration for a further period of one year from the date of its expiry unless, as a result of an inspection carried out on the premises by an inspector of the Minister, he is satisfied that the applicant has failed to comply with any of the requirements specified in Part II of Schedule 2 relating to the use of those premises as a hatchery.

(9) The provisions of paragraph (1) above shall not apply in the case of any premises used solely for the incubation or hatching of eggs from which chicks are sold or supplied for use in the manufacture of vaccines or for research or other scientific purposes.

Duties of registered persons in respect of breeding flocks and hatcheries

5. It shall be the duty of a person whose name is entered in the Breeding Flocks Register or in the Hatcheries Register in respect of any premises to ensure that the requirements specified in Part I of Schedule 2 relating to the keeping of a breeding flock on those premises or, as the case may be, the requirements specified in Part II of that Schedule relating to the use of those premises as a hatchery are complied with.

Taking of samples from breeding flocks for bacteriological testing for salmonella

6. It shall be the duty of a person whose name is entered in the Breeding Flocks Register in respect of any premises on which a breeding flock is kept by him to ensure that—

- (a) samples are taken in respect of the flock in such manner as is specified in Part I of Schedule 3 and at such times as are so specified;
- (b) such samples are identified in such a manner as to enable the laboratory to which they are submitted to know what type of samples they are, the name of the person entered in the Breeding Flocks Register in respect of the premises on which they were taken, the address of those premises and the house (if any) on those premises from which they were taken;
- (c) such samples (other than those required to be taken under the supervision of an officer of the Minister) are dispatched, within 48 hours of being taken, or, in the case of samples required to be taken over a period of 4 consecutive days, within 48 hours of the end of that period, to a laboratory for testing (at his expense) for the presence of salmonella in accordance with an appropriate bacteriological method set out in Part III of that Schedule; and
- (d) in the case of samples required to be taken under the supervision of an officer of the Minister they are given to him after being so taken for testing by the Minister for salmonella in accordance with such a bacteriological method.

Taking of blood samples from domestic fowls in breeding flocks for serological testing for *Salmonella pullorum*

7. It shall be the duty of a person whose name is entered in the Breeding Flocks Register in respect of any premises on which a breeding flock containing any domestic fowls is kept by him to use his best endeavours so as to ensure that—

- (a) blood samples are taken from the fowls in such manner as is specified in Part I of Schedule 4 and at such times as are so specified; and
- (b) such samples are tested (at his expense) by or under the supervision of an officer of the Minister for the presence of *Salmonella pullorum* in accordance with the serological method set out in Part II of that Schedule.

Taking of samples from hatcheries for bacteriological testing for salmonella

8.—(1) It shall be the duty of a person whose name is entered in the Hatcheries Register in respect of any premises used by him as a hatchery to ensure that—

- (a) samples are taken from the hatchery in such manner as is specified in Part II of Schedule 3 and at such times as are so specified;
- (b) such samples are identified in such a manner as to enable the laboratory to which they are submitted to know what type of samples they are, the name of the person entered in the Hatcheries Register in respect of the premises on which they were taken, the address of those premises and the address of the premises from which the hatching eggs from which the samples were obtained were supplied to the hatchery;
- (c) such samples (other than those required to be taken under the supervision of an officer of the Minister) are dispatched, within 48 hours of being taken, to a laboratory for testing (at his expense) for the presence of salmonella in accordance with an appropriate bacteriological method set out in Part III of that Schedule; and
- (d) in the case of samples required to be taken under the supervision of an officer of the Minister, they are given to him after being so taken for testing by the Minister for salmonella in accordance with such a bacteriological method.

Bacteriological testing of samples from breeding flocks and hatcheries and reporting of results of tests

9.—(1) It shall be the duty of the person in charge of a laboratory to which a sample has been submitted under article 6(c) or 8(c) to ensure that—

- (a) the sample is tested for the presence of salmonella in accordance with an appropriate bacteriological method set out in Part III of Schedule 3;
- (b) the result of such a test is reported in writing as soon as practicable to the person who submitted the sample; and
- (c) where, as a result of an examination carried out in accordance with such a bacteriological method, salmonella is isolated from the sample, that a subculture is sent to the Lasswade Veterinary Laboratory of the Ministry situated at Penicuik, Midlothian, Scotland or a Veterinary Investigation Centre of the Ministry in England or Wales.

(2) If a person to whom a report is made under paragraph (1)(b) above is not the person whose name is entered in the Breeding Flocks Register or the Hatcheries Register (as the case may be) in respect of the premises on which the sample was taken, he shall immediately pass that report to the person so registered.

Tampering with samples

10.—(1) No person shall treat or otherwise tamper with any sample which has been taken for the purposes of this Order.

(2) For the purposes of this article a person shall be deemed to have treated a sample if he does anything in relation to it which is likely to affect the result of the test required to be carried out under this Order.

Keeping of records

11. A person whose name is entered in the Breeding Flocks Register or in the Hatcheries Register in respect of any premises shall—

- (a) keep a record containing the information specified in Part I of Schedule 5 of any sample taken in respect of the breeding flock or hatchery (as the case may be) in accordance with article 6(a) or 8(a) (as appropriate);
- (b) keep a record of the result of any test carried out on a sample in accordance with article 6(c) or 8(c) (as appropriate) which has been reported to him in accordance with article 9;
- (c) keep a record containing the information specified in Part II of Schedule 5 of tests carried out on any day on blood samples in accordance with article 7(b);
- (d) keep a record containing the information specified in Part III of Schedule 5 of the movement of any poultry, chicks or eggs onto and off the premises on which the breeding flock is kept;

- (e) keep a record containing the information specified in Part IV of Schedule 5 of the movement of any eggs onto and off the premises used as a hatchery and of the movement of any chicks off such premises;
- (f) retain any such record for a period of 1 year from the date on which the sample was taken, or from the date of the test or from the date on which the movement took place (as the case may be);
- (g) produce any such record to an inspector or officer of the appropriate Minister on demand being made by such person at any reasonable time during that period and allow a copy of it or an extract from it to be taken.

Prohibition on vaccination

12. No person shall vaccinate any poultry with any vaccine which is likely to affect the result of any test carried out under this Order on any sample taken from the poultry, except under the authority of a licence issued by a veterinary inspector and in accordance with any conditions subject to which the licence is issued.

Exemption

13.—(1) The appropriate Minister may, if he thinks it expedient to do so, issue a certificate exempting a person from any of the requirements of article 6, 7 or 8.

(2) A certificate issued under paragraph (1) above may be issued subject to such conditions as the appropriate Minister may think fit for preventing the spread of salmonella.

Inspections of premises

14. The Minister may, before the appropriate Minister enters the name of any person in the Breeding Flocks Register or in the Hatcheries Register in respect of any premises or before the appropriate Minister renews the registration of a person in either of those registers in respect of any premises, carry out such inspections, examinations and tests as he considers necessary for the purpose of ascertaining whether any of the grounds referred to in this Order exist for the appropriate Minister to refuse to enter the name of a person in either of those registers or to refuse to renew any such registration.

Public inspection of registers

15. The appropriate Minister shall make available for inspection at any reasonable time the names of persons who are for the time being entered in the Breeding Flocks Register or the Hatcheries Register and the addresses of the premises in respect of which they are so registered.

Powers of Ministry inspectors in cases of default

16. If any person fails to take any action required to be taken by him under any of the provisions of this Order, an inspector of the Minister may, without prejudice to any proceedings arising out of such default, take, or cause to be taken, such action and the amount of any expenses reasonably incurred by him in doing so shall be recoverable by the appropriate Minister as a civil debt from the person in default.

Offences

17. Any person who, without lawful authority or excuse, proof of which shall lie on him,—

- (a) contravenes or fails to comply with any provision of this Order or with any provision of a licence or certificate issued under this Order; or
 - (b) fails to comply with any condition of any such licence or certificate,
- commits an offence against the Act.

Local authority to enforce Order

18. The provisions of this Order shall, except where otherwise expressly provided, be executed and enforced by the local authority.

In Witness whereof the Official Seal of the Minister of Agriculture, Fisheries and Food is hereunto affixed on 19th October 1989.



John Gummer
Minister of Agriculture, Fisheries and Food

18th October 1989

Sanderson of Bowden
Minister of State, Scottish Office

18th October 1989

Peter Walker
Secretary of State for Wales

SCHEDULE 1

Article 3(4)

PART I

PARTICULARS TO BE NOTIFIED TO THE APPROPRIATE MINISTER FOR THE PURPOSES OF ARTICLE 3(4)

- (i) The name, address and telephone number of the applicant
- (ii) The address and telephone number of the premises on which the flock is to be kept
- (iii) The name of the person in charge of the premises on which the flock is to be kept (if not the applicant)
- (iv) The species of birds in the flock.
- (v) The approximate number of birds in the flock.

PART II

Article 4(4)

PARTICULARS TO BE NOTIFIED TO THE APPROPRIATE MINISTER FOR THE PURPOSES OF ARTICLE 4(4)

- (i) The name, address and telephone number of the applicant
- (ii) The address and telephone number of the premises which are to be used as a hatchery
- (iii) The name of the person in charge of the premises which are to be used as a hatchery (if not the applicant)
- (iv) The incubator capacity of the premises which are to be used as a hatchery and the species of birds to be hatched there

SCHEDULE 2

Article 5

PART I

REQUIREMENTS RELATING TO THE KEEPING OF A BREEDING FLOCK

1. A person whose name is entered in the Breeding Flocks Register in respect of any premises on which a breeding flock is kept by him ("a registered person") shall ensure that the premises are located and constructed or adapted so as to be suitable for all operations carried out on those premises in connection with the flock and so as to enable the provisions of paragraph 2 below to be complied with on those premises
2. A registered person shall ensure that on the premises on which the breeding flock is kept—
 - (1) effective measures are taken so as to ensure that—
 - (a) poultry houses and buildings or parts of buildings used to store eggs are not infested by vermin; and

- (b) poultry houses in which poultry are housed permanently throughout their rearing or laying periods are not accessible to any other birds at any time;
 - (2) domestic animals are not permitted access to poultry houses;
 - (3) footbaths containing an approved disinfectant are maintained outside each house and the disinfectant is renewed as necessary and, in any event, at least once every 7 days;
 - (4) no person enters a poultry house unless he is wearing disposable overall clothing and boots or overall clothing and boots which are capable of being cleansed and disinfected or overall clothing which is capable of being laundered and boots which are capable of being cleansed and disinfected;
 - (5) no person leaves a poultry house without immediately cleansing and disinfecting his boots and washing his hands;
 - (6) after a poultry house has been depopulated of poultry all manure is removed from it and the house is cleansed and disinfected with an approved disinfectant;
 - (7) eggs are collected from the flock at least twice a day or, in the case of a flock consisting of ducks or geese, at least once a day;
 - (8) all broken, leaking, dirty and dented eggs are collected in separate containers from other eggs;
 - (9) no broken, leaking, dirty or dented eggs are incubated;
 - (10) all eggs intended for incubation are sanitised;
 - (11) all eggs which have been sanitised are stored in clean, dust-free rooms to which poultry are not permitted access and which are used exclusively for this purpose and kept at a temperature of 13°-15°C (55°-60°F) and at a relative humidity of 70-80%; and
 - (12) all equipment used for catching or transporting poultry is cleansed and disinfected with an approved disinfectant before each occasion on which it is used.
3. A registered person shall ensure that an inspector of the Minister is permitted, on demand at any reasonable time, to enter the premises on which the flock is kept in order to ascertain whether the requirements specified in paragraph 1 or 2 above are being complied with on the premises.

PART II

Article 5

REQUIREMENTS RELATING TO THE USE OF ANY PREMISES AS A HATCHERY

1. A person whose name is entered in the Hatcheries Register in respect of any premises used by him as a hatchery ("a registered person") shall ensure that the premises are located and constructed or adapted so as to be suitable for all operations carried out on those premises and so as to enable the provisions of paragraph 2 below to be complied with on those premises and, in particular, a registered person shall ensure that those premises are constructed so that-
- (a) a one way system for the movement of eggs and chicks is operated; and
 - (b) a separate ventilation system exists for each work area; or
 - (c) the airflow is in the same direction as the movement of eggs and chicks.
2. A registered person shall ensure that on the premises used as a hatchery-
- (1) broilers and stock intended for the production of eggs for human consumption are hatched separately from each other;
 - (2) pedigree, grandparent and parent stock are hatched separately from each other and from stock intended for the production of eggs for human consumption or meat;
 - (3) eggs of different species of birds are hatched separately from each other;
 - (4) eggs are sanitised before incubation;
 - (5) chick sexing tables and equipment are cleansed and disinfected between hatches using an approved disinfectant;
 - (6) all equipment which is to be returned to individual premises on which breeding flocks are kept is cleansed and disinfected with an approved disinfectant before it is returned;
 - (7) all vehicles used for transporting equipment, eggs or chicks are cleansed and disinfected with an approved disinfectant before each occasion on which they are used;
 - (8) hatching rooms, take off rooms, sexing rooms, holding rooms and dispatch rooms are cleansed and disinfected between hatches, and that all other rooms are cleansed and disinfected with an approved disinfectant each week;
 - (9) all equipment used for vaccinating birds is cleansed and disinfected with an approved disinfectant, or otherwise sterilised according to the manufacturers' instructions between hatches.

3. A registered person shall ensure that an inspector of the Minister is permitted, on demand at any reasonable time, to enter the premises used as a hatchery in order to ascertain whether the requirements specified in paragraphs 1 and 2 above are being complied with on the premises.

SCHEDULE 3

Article 6(a)

PART I

SAMPLES TO BE TAKEN FROM BREEDING FLOCKS FOR BACTERIOLOGICAL TESTING

1. Except as otherwise provided in paragraph 2 below, the number of samples to be taken shall be as follows—

<i>Number of birds kept in a house or, on premises on which birds have free access to more than one house, number of birds in each group of houses on such premises</i>	<i>Number of samples to be taken from that house or group of houses on those premises</i>
25- 29	20
30- 39	25
40- 49	30
50- 59	35
60- 89	40
90-199	50
200-499	55
500 or more	60

2. The samples to be taken shall comprise—

- (a) one chick box liner, up to a maximum of 10, for every 500 chicks delivered from each hatchery to any rearing premises on any day, such samples to be taken on the day of the arrival of the chicks there;
- (b) the carcasses of all chicks, up to a maximum of 60, from each hatchery, which are dead on arrival at any rearing premises, such samples to be taken on the day of the arrival of the chicks there;
- (c) the carcasses of all birds, up to a maximum of 60, which die or are culled within 4 days of their arrival at any rearing premises or, in the case of birds hatched on any rearing premises, within 4 days of being hatched;
- (d) the carcasses of all birds, up to a maximum of 60, which die or are culled over a period of 4 consecutive days when between 3 and 5 weeks of age, which samples shall be taken over such period, except that, if the total number of such carcasses taken during such period is less than the number of samples specified in the second column of the table in paragraph 1 above the difference shall be made up by—
 - (i) a composite faeces sample consisting of an equivalent number of individual samples, or
 - (ii) an equivalent number of cloacal swabs, taken at the rate of one swab from each bird;
- (e) the carcasses of all birds at grandparent level and above, up to a maximum of 60, which die or are culled over a period of 4 consecutive days when between 8 and 12 weeks of age, which samples shall be taken over such period, except that, if the total number of carcasses taken during such period is less than the number of samples specified in the second column of the table in paragraph 1 above the difference shall be made up by—
 - (i) a composite faeces sample consisting of an equivalent number of individual samples, or
 - (ii) an equivalent number of cloacal swabs, taken at the rate of one swab from each bird;
- (f) (i) the carcasses of all birds, up to a maximum of 60, which die or are culled over a period of 4 consecutive days taken either 3 to 5 weeks before the surviving birds are transferred to laying accommodation, or when the birds are between the ages of 16 and 22 weeks in the case of domestic fowls, 18 and 22 weeks in the case of ducks, 26 and 30 weeks in the case of turkeys and 30 and 36 weeks in the case of geese, whichever occurs later, which samples shall be taken over such a period, except that, if the total number of carcasses taken during such period is less than the number of samples specified in the second column of the table in paragraph 1 above the difference shall be made up by—

- a composite faeces sample consisting of an equivalent number of individual samples, or
- an equivalent number of cloacal swabs, taken at the rate of one swab from each bird; and
- (ii) a single composite sample of 50 grams of dust consisting of equal amounts of dust from each ventilation outlet in the house;
- (g) in the case of birds whose eggs are hatched at a hatchery with a total incubator capacity of less than 1,000 eggs or whose eggs are not being hatched-
 - (i) a composite faeces sample consisting of a number of individual samples calculated in accordance with the said table, or
 - (ii) a number of cloacal swabs, calculated in accordance with the table in paragraph 1 above and taken at the rate of one swab from each bird;
 such samples being taken within 1 week of the birds attaining 26 and 30 weeks of age and at 8 week intervals thereafter;
- (h) after a house has been depopulated of poultry and before it is restocked the following samples shall be taken-
 - (i) an individual large cotton bud swab moistened with sterile Buffered Peptone Water (BPW)(a) from each fan housing;
 - (ii) an individual large cotton bud swab moistened with sterile BPW from-
 - each of the four corners of the house at floor level,
 - the centre of each of the four walls of the house at floor level, and
 - two crevices in the house;
 - (iii) each food weighing hopper or each food dispensing hopper within each house; and
 - (i) in the case of any nest boxes which have been removed from a house, before they are put back in the house swabs moistened with sterile BPW shall be taken from the interior of such boxes at the rate of one in 20.

3. In the case of domestic fowls, the samples referred to in paragraph 2(f) above, other than the carcasses of birds, shall be taken under the supervision of an officer of the Minister.

4. Where any samples are taken over a period of 4 consecutive days such samples shall be stored in a refrigerator at between 1°C and 4°C until they are dispatched to a laboratory and, in the case of any other samples, where they cannot be dispatched to a laboratory within 24 hours of being taken they shall be stored in a refrigerator at that temperature until so dispatched.

PART II

Article 8(a)

SAMPLES TO BE TAKEN FROM HATCHERIES FOR BACTERIOLOGICAL TESTING

1. The samples to be taken shall comprise-
 - (a) composite samples of meconium taken from 250 chicks every 7 days, one such sample being taken from those chicks hatched from eggs supplied to the hatchery from any particular premises; or
 - (b) samples comprising-
 - (i) the carcasses of all chicks which are dead in the shells of eggs supplied to the hatchery from any particular premises, and
 - (ii) the carcasses of all chicks hatched from eggs supplied to the hatchery from any particular premises and which have been culled,
 such samples being taken every 7 days, up to a maximum of 50 in total, and
 - (c) the carcasses of all chicks hatched from eggs supplied to the hatchery from any particular premises and which have been culled, up to a maximum of 50, such samples to be taken every 28 days under the supervision of an officer of the Minister.
2. Samples taken for the purposes of paragraph 1(a) and (b) above shall be taken, every 28 days, under the supervision of an officer of the Minister.
3. Where any samples cannot be dispatched to a laboratory within 24 hours of being taken they shall be stored in a refrigerator at between 1°C and 4°C until so dispatched.

(a) Buffered Peptone Water - Edcl and Kapelmacher (1973) (Commercially available as Oxoid CM 509, Lab M46 or equivalent)

BACTERIOLOGICAL METHODS FOR TESTING FOR SALMONELLA

1. Bacteriological method (Rappaports) for the detection of salmonella in chick box liners, cloacal swabs, composite faeces samples, meconium samples, carcasses, dust and environmental swabs (ie: swabs taken from fan housings, walls, floors, crevices, food weighing and dispensing hoppers and nest boxes).

Samples submitted for testing for the presence of salmonella shall be examined in the following prescribed manner on consecutive days and, where a laboratory at which samples have been received for testing on any day is unable to commence such an examination on that day, the samples shall be stored in a refrigerator at between 1°C and 4°C until required for examination.

Day 1

- (a) Chick box liners: a one gram portion shall be taken from a soiled area on each liner and the portions from separate liners shall be bulked together and placed in Buffered Peptone Water (BPW)(a), at the rate of 1 gram of liner in 10 ml of BPW up to a maximum of 10 grams in 100 ml of BPW.
- (b) Composite faeces and meconium samples: the samples shall be thoroughly mixed and a sub-sample weighing not more than 10 grams shall be placed in BPW at the rate of 1 gm sample to 10 ml BPW to a maximum of 10 grams in 100 ml BPW.
- (c) Cloacal swabs: Cloacal swabs shall be bulked together in batches and placed in BPW at the rate of 1 swab to 4 ml BPW up to a maximum of 30 swabs in 120 ml BPW.
- (d) Dust samples: the composite sample shall be thoroughly mixed and a sub-sample of 10 grams shall be placed in 225 ml of BPW.
- (e) Environmental swabs shall be bulked together in batches and placed in BPW at the rate of 1 swab to 10 ml BPW up to a maximum of 10 swabs in 100 ml BPW.
- (f) Carcasses of birds: the following organs shall be removed from the carcasses of birds—
 - (i) from chicks – samples of the yolk sac, liver and terminal intestines (to include portions of small intestines, large intestine and caecal tonsil),
 - (ii) from birds (other than chicks) – samples of liver and terminal intestines (to include portions of small intestines, large intestine and caecal tonsil).

The samples of organs taken from the carcasses of birds submitted shall then be bulked together and placed in BPW at the rate of 1 gram of bulked tissue in 10 ml BPW up to a maximum of 10 grams of tissue in 100 ml BPW.

The inoculated BPW shall then be incubated at 37°C for 18–24 hours.

Day 2

0.1 ml from the incubated BPW shall be inoculated into 10 ml of Rappaports Vassiliadis (RV) broth (b) and incubated at 42.5°C ± 0.5°C for 18–24 hours.

Day 3

The RV broth shall be plated out on to two plates of Brilliant Green Agar (BGA) (d) using a 2.5 mm diameter loop. The BGA plates shall be inoculated with a droplet taken from the edge of the surface of the fluid and drawing the loop over the whole of one plate in a zigzag pattern and continuing to the second plate without recharging the loop. The space between the loop streaks shall be 0.5–1.0 cm. The plates shall be incubated at 37°C for 18–24 hours, and the RV broth reincubated at 42.5°C ± 0.5°C for a further 18–24 hours.

Day 4

- (i) The plates of BGA shall be examined and a minimum of 3 colonies from the plates showing suspicion of salmonella growth shall be subcultured on to a blood agar plate and a MacConkey agar plate and into biochemical composite media or equivalent. These media shall be incubated at 37°C for 18–24 hours.
- (ii) The reincubated RV broth shall be plated out, and the plates incubated, as described in Day 3.

Day 5

- (i) The incubated plates and composite media or equivalent shall be examined and the findings recorded, discarding cultures which are obviously not salmonella. Slide serological tests shall be performed using salmonella polyvalent "O" (Groups A–S) and polyvalent "H" (phase 1 and 2) agglutinating sera on selected suspect colonies

collected from the blood agar or MacConkey plates. If reactions occur with one or both sera, the colonies shall be typed to Group level by slide serology.

- (ii) The plates of BGA prepared at Day 4 (ii) shall be examined and further action taken as described in Day 4 (i) and Day 5 (i).

2. Bacteriological method (Selenite) for the detection of salmonella in chick box liners, cloacal swabs, composite faeces samples, meconium samples and carcasses.

Samples submitted for testing for the presence of salmonella shall be examined in the following prescribed manner on consecutive days and, where a laboratory at which samples have been received for testing on any day is unable to commence such an examination on that day, the samples shall be stored in a refrigerator at between 1°C and 4°C until required for examination.

Day 1

- (a) Chick box liners: a one gram portion shall be taken from a soiled area on each liner and the portions from separate liners shall be bulked together and placed in Selenite F broth (c) at the rate of 1 gram of liner to 10 ml broth up to a maximum of 10 grams of liner in 100 ml broth.
- (b) Composite faeces and meconium samples: the sample shall be thoroughly mixed and a sub-sample weighing not more than 10 grams shall be placed in Selenite F broth at the rate of 1 gram of faeces to 10 ml broth up to a maximum of 10 grams of faeces in 100 ml broth.
- (c) Cloacal swabs: cloacal swabs shall be bulked together in batches and placed in Selenite F broth at the rate of 1 swab to 4 ml broth up to a maximum of 30 swabs in 120 ml broth.
- (d) Carcasses of birds: the following organs shall be removed from the carcasses of birds—
- (i) from chicks – samples of the yolk sac, liver and terminal intestines (to include portions of small intestines, large intestine and caecal tonsil).
- (ii) from birds (other than chicks) – samples of liver and terminal intestines (to include portions of small intestines, large intestine and caecal tonsil).

The samples of organs taken from the carcasses of birds submitted shall then be bulked together and placed in Selenite F broth at the rate of 1 gram of bulked tissue in 10 ml of broth up to a maximum of 10 grams of tissue in 100 ml broth.

The inoculated Selenite F broth shall then be incubated at 37°C for 18–24 hours.

Day 2

- (i) The Selenite F broth shall be plated out on to two plates of Brilliant Green Agar (BGA) (d) using a 2.5 mm diameter loop. The BGA plates shall be inoculated with a droplet taken from the edge of the surface of the fluid and drawing the loop over the whole of one plate in a zigzag pattern and continuing to the second plate without recharging the loop. The space between the loopstreaks shall be 0.5 cm–1.0 cm. The plates shall be incubated at 37°C for 18–24 hours.
- (ii) The Selenite F broth shall then be reincubated at 37°C for a further 18–24 hours.

Day 3

- (i) The plates of BGA shall be examined and a minimum of 3 colonies from the plates showing suspicion of salmonella growth shall be subcultured on to a blood agar plate and a MacConkey agar plate and into biochemical composite media or equivalent.

These media shall be incubated at 37°C for 18–24 hours.

- (ii) The reincubated Selenite F broth shall be plated out and incubated as described in Day 2 (i).

Day 4

- (i) The incubated plates and composite media or equivalent shall be examined and the findings recorded, discarding cultures which are obviously not salmonella. Slide serological tests shall be performed using salmonella polyvalent "O" (Groups A–S) and polyvalent "H" (phase 1 and 2) agglutinating sera on selected suspect colonies collected from the blood agar or MacConkey plates. If reactions occur with one or both sera, the colonies shall be typed to Group level by slide serology.
- (ii) The plates of BGS prepared at Day 3 (ii) shall be examined and further action taken as described in Day 3 (i) and Day 4 (i).

(a) Buffered Peptone Water – Edel and Kampelmacher (1973) (commercially available as Oxoid CM 509, Lab M46 or equivalent)

(b) Rappaport Vassiliadis (RV) Broth – Vassiliadis et al (1976) (commercially available as Oxoid CM 669 or equivalent)

- (c) Selenite F broth – Liefson (1936) (commercially available as Oxoid CM 395 and L121, Lab M44a and 44b or equivalent)
(a), (b) and (c) should be reconstituted according to the manufacturer's instructions.
- (d) Brilliant Green Agar (Modified) – Edel and Kampelmacher (1969) (commercially available as Oxoid CM 329, Lab M34 or equivalent)
The agar should be reconstituted according to the manufacturer's instructions and poured into 9 cm diameter plates.

References:

- Liefson E. (1936) *American Journal of Hygiene* 24, 423-432.
- Edel, W. & Kampelmacher, E.H. (1969) *Bulletin of the World Health Organisation* 41, 297-306.
- Edel W. & Kampelmacher E.H. (1973) *Bulletin of the World Health Organisation* 48, 167-174
- Anon (1969) ISO 6579 International Organisation for Standardisation, Geneva
- Vassiliadis, P., Pateraki, E., Papaiconomou, N., Papadakis, J.A.,
and Trichopoulos, D. (1976) *Annales de Microbiologie (Institut Pasteur)* 127B, 195-200

SCHEDULE 4

Article 7(a)

PART I

BLOOD SAMPLES TO BE TAKEN FOR SEROLOGICAL TESTING FOR SALMONELLA PULLORUM

1. Blood samples shall be taken, by or under the supervision of an officer of the Minister, from domestic fowls between 3 and 5 weeks before they are transferred to laying accommodation or when they are between 16 and 22 weeks of age, whichever occurs later.
2. The number of birds from which blood samples shall be taken shall be as follows—
(a) in the case of birds at grandparent level and above, all birds; and
(b) in the case of birds at parent level, a number calculated in accordance with the following table—

Number of birds at parent level required to be sampled for testing for *Salmonella pullorum*

<i>Number of birds kept in a house or, on premises on which birds have free access to more than one house, number of birds in each group of houses on such premises</i>	<i>Number of birds to be sampled in that house or in that group of houses on those premises</i>
300 or less	All birds up to 150
301 - 400	160
401 - 4000	170
4001 or more	180

3. The samples shall comprise 0.02 ml of blood taken from a wing vein of a bird by pricking with a suitable needle

SEROLOGICAL METHOD FOR TESTING FOR SALMONELLA PULLORUM

The rapid plate whole blood test shall be used for the testing of blood samples for *Salmonella pullorum* which tests shall be carried out as follows—

- (1) 0.02 ml of blood taken from a wing vein of a bird, after pricking with a suitable needle, shall be placed on a white ceramic tile using a loop of the appropriate size.
- (2) 0.04 ml of polyvalent crystal violet stained *Salmonella pullorum* antigen(a) shall be added to the blood and mixed with it.
- (3) The tile shall be rocked gently for 2 minutes after which time the test shall be read.
- (4) All bleeding needles and loops must be washed in a normal saline solution(b) after each bird has been sampled and tested which solution must be renewed after every 200 birds have been sampled and tested.

SCHEDULE 5

Article 11(a)

PART I

RECORDS OF SAMPLES TAKEN IN RESPECT OF A BREEDING FLOCK OR A HATCHERY

A person whose name is entered in the Breeding Flocks Register in respect of any premises on which a breeding flock is kept by him or whose name is entered in the Hatcheries Register in respect of any premises used by him as a hatchery shall keep a record of samples taken in respect of the flock or hatchery (as the case may be) containing the following information—

- (i) the date on which the sample was taken;
- (ii) a description of the type of sample taken; and
- (iii) in the case of samples taken in respect of a breeding flock, the identity of the house or group of houses from which the samples were taken and, in the case of samples taken from a hatchery, the address of the premises from which the hatching eggs from which the samples were obtained were supplied to the hatchery

PART II

Article 11(c)

RECORDS OF TESTS CARRIED OUT ON BLOOD SAMPLES FROM BREEDING FLOCKS

A person whose name is entered in the Breeding Flocks Register in respect of any premises on which a breeding flock is kept by him shall keep a record of tests carried out on any day on blood samples taken from domestic fowls in the flock which record shall contain the following information—

- (i) the date of the tests;
- (ii) the number of birds from which blood samples were tested on that day; and
- (iii) the number of birds giving a positive reaction to tests for *Salmonella pullorum* carried out on that day

PART III

Article 11(d)

RECORDS OF THE MOVEMENT OF POULTRY, CHICKS AND EGGS ONTO AND OFF ANY PREMISES ON WHICH A BREEDING FLOCK IS KEPT

A person whose name is entered in the Breeding Flocks Register in respect of any premises on which a breeding flock is kept shall keep a record of the movement of any poultry, chicks and eggs onto and off such premises which record shall contain the following information—

- (i) the date of the movement;

(a) *S. pullorum* antigen must contain standard and variant strains of *S. pullorum* stained with crystal violet and standardised against international standard sera raised against the standard and variant strains (O.I.E. 1986)

(b) Normal saline solution is prepared by dissolving saline tablets in water according to the manufacturers instructions.

Reference: O.I.E. (1986) International Zoosanitary Code 5th Edition, 362-364 (updated May 1988 as International Animal Health Code, updated pages 45 to 47).

- (ii) the number of poultry, chicks or eggs moved;
- (iii) the identity of the house or group of houses in which any poultry, chicks or eggs moved onto the premises were placed or from which any poultry, chicks or eggs were moved off the premises;
- (iv) in the case of any poultry, chicks or eggs moved onto the premises, the address from which they were brought there; and
- (v) in the case of any poultry, chicks or eggs moved off the premises, the address of the premises to which they were moved.

PART IV

Article 11(e)

RECORDS OF THE MOVEMENT OF EGGS ONTO, AND OFF AND OF THE MOVEMENT OF CHICKS OFF, ANY PREMISES WHICH ARE USED AS A HATCHERY

A person whose name is entered in the Hatcheries Register in respect of any premises used by him as a hatchery shall keep a record of the movement of any eggs onto and off, and of the movement of any chicks off, such premises, which record shall contain the following information—

- (i) the date of the movement;
- (ii) in the case of the movement of any eggs onto the premises, the address of the premises from which they were moved and the number of eggs moved;
- (iii) in the case of the movement of any eggs or chicks off the premises the address of the premises to which they were moved and the number of eggs or chicks moved.

EXPLANATORY NOTE

(This note is not part of the Order)

This Order prohibits (with two exceptions) a person from keeping a breeding flock on any premises or from using any premises as a hatchery unless his name is entered in the Breeding Flocks Register or in the Hatcheries Register in respect of those premises, both such registers being kept by the appropriate Minister (the Minister of Agriculture, Fisheries and Food or the Secretary of State for Scotland or Wales) for the purposes of the Order. Such registration will remain in force for a period of one year and will thereafter be renewed annually by the appropriate Minister unless following an inspection carried out on the premises, he is satisfied that the registered person has failed to comply with certain specified requirements relating to the keeping of a breeding flock on the premises or to the use of the premises as a hatchery (articles 3 and 4 and Schedules 1 and 2).

These prohibitions do not apply in the case of (i) a breeding flock consisting of birds which are kept solely for the production of hatching eggs or chicks for use in the manufacture of vaccines or for research or other scientific purposes or (ii) any premises which are used solely for the incubation or hatching of eggs from which chicks are sold or supplied for such use or for any such purposes (articles 3(9) and 4(9)).

The Order requires a registered person to ensure that samples are taken in respect of the breeding flock or hatchery and are submitted to a laboratory for testing for the presence of salmonella (other than samples taken under the supervision of an officer of the Minister which are required to be given to that officer for testing for this purpose) (articles 6 and 8 and Schedule 3).

A person registered in respect of a breeding flock containing any domestic fowls is also required to ensure that blood samples are taken from the fowls for testing, by or under the supervision of an officer of the Minister, for *Salmonella pullorum* (article 7 and Schedule 4).

The Order also requires the person in charge of a laboratory to which any sample taken in respect of a breeding flock or a hatchery has been submitted to ensure that the test is carried out in a required manner and that the result of the test is reported to the

person who submitted the sample and who, if he is not the person registered in respect of the flock or hatchery in respect of which the sample was taken, is required to pass the report to the person so registered (article 9).

The Order also contains provisions which—

- (1) prohibit any tampering with samples (article 10);
- (2) require registered persons to keep records of samples taken and, of the results of tests on samples and to keep records of the movement of poultry, chicks and eggs onto and off any premises on which a breeding flock is kept or which are used as a hatchery (article 11);
- (3) prohibit (except under licence) the vaccination of poultry with any vaccine which is likely to affect the result of any test carried out under the Order on any sample taken from the poultry (article 12); and
- (4) enable the appropriate Minister to issue a certificate exempting any person from the sampling and testing provisions of the Order subject to such conditions as the appropriate Minister may think fit for preventing the spread of salmonella (article 13).

The provision requiring the registration of a person who keeps a breeding flock on any premises or who uses any premises as a hatchery shall come into force on 2nd April 1990. Prior to that date the duties of a registered person under the Order shall be the duties of a person who is keeping a breeding flock on any premises or who is using any premises as a hatchery and whose name will be required to be entered in the Breeding Flocks Register or in the Hatcheries Register in respect of the premises when those provisions come into force (articles 1(2) and 2(2)).

Until 1st June 1990 samples (other than blood samples) taken under the Order are required to be tested at laboratories which have the necessary facilities and personnel for carrying out the tests in accordance with the Order and after that date the samples are required to be tested at laboratories authorised in writing by the Minister for this purpose (article 2(4)).

For the purposes of this Order "breeding flock" means any flock of poultry consisting of not less than 25 birds which are kept (or are being reared) for the production of hatching eggs or birds for sale or supply for breeding purposes or for the production of eggs or for meat and "hatchery" means any premises, with a total incubator capacity of not less than 1,000 eggs, on which the eggs of poultry are incubated or hatched and from which chicks are sold or supplied (article 2(1)).

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 STATUTORY INSTRUMENTS

1990 No. 347

ANIMALS

ANIMAL HEALTH

The Poultry Breeding Flocks and Hatcheries (Registration and Testing) (Amendment) Order 1990

Made - 13th February 1990

Coming into force 19th February 1990

The Minister of Agriculture, Fisheries and Food, the Secretary of State for Scotland and the Secretary of State for Wales, acting jointly, in exercise of the powers conferred on them by sections 1 and 86(1) of the Animal Health Act 1981^(a) and of all other powers enabling them in that behalf, hereby make the following Order:

Title and commencement

1. This Order may be cited as the Poultry Breeding Flocks and Hatcheries (Registration and Testing) (Amendment) Order 1990 and shall come into force on 19th February 1990.

Amendment

2. The Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989^(b) shall be amended as follows—

- (a) in paragraph (2) of article 2 (interpretation) the words from "and whose name will be required to be entered in the Breeding Flocks Register or in the Hatcheries Register" to the end of that paragraph shall be omitted;
- (b) article 15 (public inspection of registers) shall be omitted;
- (c) in Part 1 of Schedule 3 (samples to be taken from breeding flocks for bacteriological testing)—

(i) in paragraph 1 for the first entry in the table there shall be substituted the following entry—

"1-29 A number equal to the total number of birds up to a maximum of 20 birds";

(ii) in paragraphs 2(d) and (e) for the words—

"the difference shall be made up by—

- (a) a composite faeces sample consisting of an equivalent number of individual samples, or
- (b) an equivalent number of cloacal swabs, taken at the rate of one swab from each bird;" there shall be substituted the following—

"the deficit shall be made up by—

- (i) a composite faeces sample consisting of a number of individual samples equal to that deficit, or

^(a) 1981 c.22, as applied by S.I. 1989/285, section 86(1) contains a definition of "the Ministers" relevant to the exercise of the statutory powers under which this Order is made.

^(b) S.I. 1989/1963.

- (ii) a number of cloacal swabs, taken at the rate of one swab from each bird, equal to that deficit;" and
- (iii) in paragraph 2(f)(i) for the words "the difference shall be made up by-
 - a composite faeces sample consisting of an equivalent number of individual samples, or
 - an equivalent number of cloacal swabs, taken at the rate of one swab from each bird; and"
 there shall be substituted the following-
 - "the deficit shall be made up by-
 - a composite faeces sample consisting of a number of individual samples equal to that deficit, or
 - a number of cloacal swabs, taken at the rate of one swab from each bird, equal to that deficit; and"

In Witness whereof the Official Seal of the Minister of Agriculture, Fisheries and Food is hereunto affixed on 12th February 1990.



John Selwyn Gummer
Minister of Agriculture, Fisheries and Food

13th February 1990

Sanderson of Bowden
Minister of State, Scottish Office

13th February 1990

Peter Walker
Secretary of State for Wales

EXPLANATORY NOTE

(This note is not part of the Order)

This Order amends the Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989. The requirement for the appropriate Minister to make the Breeding Flocks Register and the Hatcheries Register available for public inspection is omitted (article 2(b)). The Order confirms that the duty of a registered person under article 6(a) of the 1989 Order concerning the taking of samples is applied even where only one bird is kept in a house (article 2(c)(i)). However, as before, the 1989 Order will require a person to be registered only if he keeps a breeding flock which consists of not less than 25 birds.

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APPENDIX A.3

The Poultry Breeding Flocks and Hatcheries Order 1993

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In
Original

STATUTORY INSTRUMENTS

1993 No. 1898

ANIMALS

ANIMAL HEALTH

The Poultry Breeding Flocks and Hatcheries Order 1993

Made 23rd July 1993

Coming into force 30th August 1993

The Minister of Agriculture, Fisheries and Food, the Secretary of State for Scotland and the Secretary of State for Wales, acting jointly, in exercise of the powers conferred on them by sections 1, 7 and 8 of the Animal Health Act 1981(a) and of all other powers enabling them in that behalf, hereby make the following Order:

Title and commencement

1. This Order may be cited as the Poultry Breeding Flocks and Hatcheries Order 1993 and shall come into force on 30th August 1993.

Interpretation

2.—(1) In this Order, unless the context otherwise requires—

"the Act" means the Animal Health Act 1981;

"breeding flock" means any flock of poultry consisting of at least 250 birds of a single species which are kept or reared on a single holding for the production of hatching eggs;

"the Breeding Flocks Register" means the register kept by the appropriate Minister under article 3(2);

"building" includes a shed and a part of a building separated from other parts of that building by a solid partition and having its own ventilation system;

"chicks" means poultry less than 72 hours old which have not been fed;

"chick box liner" means any material used to line a box or other container in which chicks are transported from a hatchery to any rearing premises;

"composite faeces sample" means a sample of faeces consisting of a number of individual samples of faeces calculated in accordance with the appropriate provisions of Part 1 of Schedule 2 each of which weighs not less than 1 gram and is taken from a site selected at random to represent the building or group of buildings on the holding from which it is taken;

"domestic fowl" means birds of the species *Gallus gallus*;

"the Hatcheries Register" means the register kept by the appropriate Minister under article 4(2);

"hatchery" means any premises, with a total incubator capacity of not less than 1,000 eggs, on which the eggs of poultry are incubated or hatched and from which chicks are sold or supplied;

"hatching eggs" means eggs intended for incubation;

"laboratory" means any laboratory authorised in writing by the Minister for the purposes of this Order which has the necessary facilities and personnel for carrying

(a) 1981 c.22, as applied by S.I. 1989/285, section 86(1)(c) contains a definition of "the Ministers".

out tests on samples mentioned in Parts I and II of Schedule 2 in accordance with the provisions of Part III of that Schedule;

"the Ministry" means the Ministry of Agriculture, Fisheries and Food;

"poultry" means domestic fowls, turkeys, geese or ducks;

"premises" includes land;

"rearing premises" means any holding on which chicks are placed for rearing as replacement breeding stock.

(2) Any reference in this Order to a numbered article or Schedule is a reference to the article or Schedule bearing that number in this Order.

(3) The provisions of this Order shall not apply in the case of a breeding flock or hatchery which is kept solely for the production of hatching eggs for use in the manufacture of vaccines or for research or other scientific purposes.

Registration of breeding flocks

3.—(1) Within 3 months of this Order coming into force, any person owning a breeding flock shall procure that his name is entered in the Breeding Flocks Register.

(2) The appropriate Minister shall keep, for the purpose of paragraph (1) above, a register of persons as being persons owning a breeding flock on a holding in respect of which their names are entered in the register.

(3) Where a person makes an application in writing to the appropriate Minister for his name to be entered in the Breeding Flocks Register in respect of any holding the appropriate Minister shall, subject to paragraph (4) below, enter his name in the Breeding Flocks Register in respect of that holding and shall issue to the applicant a certificate of such registration.

(4) A person making an application under paragraph (3) above shall supply to the appropriate Minister in his application all the particulars specified in Part I of Schedule 1.

(5) A person whose name is entered in the Breeding Flocks Register in respect of any holding shall, within 28 days of each anniversary of the date of such registration, notify the appropriate Minister in writing of any change in the particulars previously notified to him.

(6) The registration of the name of a person in the Breeding Flocks Register in respect of any holding shall remain in force for an unlimited period, except that, where a person so registered has notified the appropriate Minister in accordance with paragraph (5) above of a change in the particulars previously notified and that change consists of a reduction in the number of birds kept in the breeding flock to less than 250, the appropriate Minister shall revoke such registration unless that person informs the appropriate Minister in

writing, within 28 days of the anniversary of the date of such registration, that he anticipates that the number of such birds which will be kept by him on the holding will increase to 250 or more during the 12 months following the anniversary of such registration.

Registration of hatcheries

4.—(1) Within 3 months of this Order coming into force, any person using any premises as a hatchery shall procure that his name is entered in the Hatcheries Register in respect of those premises.

(2) The appropriate Minister shall keep, for the purpose of paragraph (1) above, a register of persons as being persons using as a hatchery any premises in respect of which their names are entered in the register.

(3) Where a person makes an application in writing to the appropriate Minister for his name to be entered in the Hatcheries Register in respect of any premises the appropriate Minister shall, subject to paragraph (4) below, enter his name in the Hatcheries Register in respect of those premises and shall issue to the applicant a certificate of such registration.

(4) A person making an application under paragraph (3) above shall supply to the appropriate Minister in his application all the particulars specified in Part II of Schedule 1.

(5) A person whose name is entered in the Hatcheries Register in respect of any premises shall, within 28 days of each anniversary of the date of such registration, notify the appropriate Minister in writing of any change in the particulars previously notified to him.

(6) The registration of the name of a person in the Hatcheries Register in respect of any premises shall remain in force for an unlimited period, except that, where a person so registered has notified the appropriate Minister in accordance with paragraph (5) above of a change in the particulars previously notified and that change consists of a reduction in the total incubator capacity of the premises to less than 1,000 eggs, the appropriate Minister shall revoke such registration unless that person informs the appropriate Minister in writing, within 28 days of the anniversary of the date of such registration, that he anticipates that the total incubator capacity of the premises will increase to 1,000 eggs or more during the 12 months following the anniversary of such registration.

Taking of samples from breeding flocks of domestic fowl for bacteriological testing for salmonella

5. It shall be the duty of a person in respect of any holding of his on which a breeding flock of domestic fowl is kept and of the person in charge of any such holding to ensure that—

- (a) samples are taken in respect of the flock in such manner as is specified in Part I of Schedule 2 and at such times as are so specified;
- (b) such samples are identified in such a manner as to enable the laboratory to which they are submitted to know what type of samples they are, the date on which they were taken, the name of the owner or person in charge of the breeding flock, the address of the holding on which the breeding flock is kept and the building (if any) on that holding from which they were taken;
- (c) such samples (other than those required to be taken under the supervision of an officer of the Minister) are dispatched, within 48 hours of being taken, to a laboratory for testing (at his expense) for the presence of salmonella in accordance with an appropriate bacteriological method set out in Part III of Schedule 2; samples shall be kept in a refrigerator at between 1°C and 4°C if they are not so dispatched within 24 hours of being taken; and
- (d) in the case of samples required to be taken under the supervision of an officer of the Minister they are given to him after being so taken for testing by the Minister for salmonella in accordance with such a bacteriological method.

Taking of samples from hatcheries for bacteriological testing for salmonella

6. It shall be the duty of a person in respect of any hatchery of his where eggs of domestic fowl are incubated or the person in charge of any such hatchery to ensure that—

- (a) samples are taken from the hatchery in such manner as is specified in Part II of Schedule 2 and at such times as are so specified;
- (b) such samples are identified in such a manner as to enable the laboratory to which they are submitted to know what type of samples they are, the date on which they were taken, the name of the person owning or in charge of the hatchery from which they were taken, the address of that hatchery and the address of the holding from which the hatching eggs from which the samples were obtained were supplied to the hatchery;
- (c) such samples (other than those required to be taken under the supervision of an officer of the Minister) are dispatched, within 48 hours of being taken, to a laboratory for testing (at his expense) for the presence of salmonella in accordance with an appropriate bacteriological method set out in Part III of Schedule 2; samples shall be kept in a refrigerator at between 1°C and 4°C if they are not so dispatched within 24 hours of being taken; and
- (d) in the case of samples required to be taken under the supervision of an officer of the Minister, they are given to him after being so taken for testing by the Minister for salmonella in accordance with such a bacteriological method.

Bacteriological testing of samples from breeding flocks and hatcheries and reporting of results of tests

7.—(1) It shall be the duty of the person in charge of a laboratory to which a sample has been submitted under article 5(c) or 6(c) to ensure that—

- (a) the sample is tested for the presence of salmonella in accordance with an appropriate bacteriological method set out in Part III of Schedule 2;
- (b) the result of such a test is reported in writing as soon as practicable to the person who submitted the sample; and
- (c) where, as a result of an examination carried out in accordance with such a bacteriological method, salmonella is isolated from the sample, that a subculture is sent to the Lasswade Veterinary Laboratory of the Ministry situated at Penicuik, Midlothian, Scotland or a Veterinary Investigation Centre of the Ministry in England or Wales.

(2) If a person to whom a report is made under paragraph (1)(b) above is not the owner of the breeding flock or the hatchery (as the case may be), he shall immediately pass that report to the owner.

Tampering with samples

8.—(1) Except as provided for in this Order, no person shall treat or otherwise tamper with any sample which has been taken for the purposes of this Order.

(2) For the purposes of this article a person shall be deemed to have treated a sample if he does anything in relation to it which is likely to affect the result of the test required to be carried out under this Order.

Keeping of records

9. A person who owns or is in charge of any breeding flock or hatchery containing, in either case, domestic fowl or their eggs shall—

- (a) keep a record containing the information specified in Part I of Schedule 3 of any sample taken in respect of the breeding flock or hatchery (as the case may be) in accordance with article 5(a) or 6(a) (as appropriate);
- (b) keep a record of the result of any test carried out on a sample in accordance with articles 5(c) or 6(c) (as appropriate) which has been reported to him in accordance with article 7;
- (c) keep a record containing the information specified in Part II of Schedule 3 of the movement of any domestic fowl or their chicks or eggs onto and off the holding on which the breeding flock is kept;
- (d) keep a record containing the information specified in Part III of Schedule 3 of the movement of any eggs of domestic fowl onto and off the premises used as a hatchery and of the movement of any chicks of domestic fowl off such premises;
- (e) retain any such record for a period of 1 year from the date on which the sample was taken, or from the date of the test or from the date on which the movement took place (as the case may be);
- (f) produce any such record to an inspector or officer of the appropriate Minister on demand being made by such person at any reasonable time during that period and allow a copy of it or an extract from it to be taken.

Prohibition on vaccination

10. No person shall vaccinate any domestic fowl with any vaccine which is likely to affect the result of any test carried out under this Order on any sample taken from the domestic fowl, except under the authority of a licence issued by a veterinary inspector and in accordance with any conditions subject to which the licence is issued.

Powers of Ministers in cases of default

11. If any person fails to take any action required to be taken by him under any of the provisions of this Order, an inspector of the Minister may, without prejudice to any proceedings arising out of such default, take, or cause to be taken, such action and the

amount of any expenses reasonably incurred by him in doing so shall be recoverable by the appropriate Minister from the person in default.

Local authority to enforce Order

12. The provisions of this Order shall, except where otherwise expressly provided, be executed and enforced by the local authority.

Revocation

13. The Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989(a) and the Poultry Breeding Flocks and Hatcheries (Registration and Testing) (Amendment) Order 1990(b) are revoked.

In witness whereof the Official Seal of the Minister of Agriculture, Fisheries and Food is hereunto affixed on 22nd July 1993.



Gillian Shephard
Minister of Agriculture, Fisheries and Food

22nd July 1993

Hector Monro
Parliamentary Under Secretary of State,
Scottish Office

23rd July 1993

John Redwood
Secretary of State for Wales

SCHEDULE I

Article 3(4)

PART I

PARTICULARS TO BE NOTIFIED TO THE APPROPRIATE MINISTER FOR THE PURPOSES OF ARTICLE 3(4)

- (i) The name, address and telephone number of the applicant, including the names of partners if a partnership and the registered number if a company.
- (ii) The address and telephone number of the holding on which the flock is to be kept.
- (iii) The name of the person in charge of the holding on which the flock is to be kept (if not the applicant).
- (iv) The species of birds in the flock.
- (v) The approximate number of birds in the flock.

PART II

Article 4(4)

PARTICULARS TO BE NOTIFIED TO THE APPROPRIATE MINISTER FOR THE PURPOSES OF ARTICLE 4(4)

- (i) The name, address and telephone number of the applicant, including the names of partners if a partnership, and the registered number if a company.
- (ii) The address and telephone number of the premises which are to be used as a hatchery.
- (iii) The name of the person in charge of the premises which are to be used as a hatchery (if not the applicant).
- (iv) The incubator capacity of the premises which are to be used as a hatchery and the species of birds to be hatched there.

(a) S.I. 1989/1963
(b) S.I. 1990/347

PART I

SAMPLES TO BE TAKEN FROM BREEDING FLOCKS OF DOMESTIC FOWL
FOR BACTERIOLOGICAL TESTING

1. The number of sites from which separate faeces samples are to be taken in order to make a composite sample shall be as follows:

<i>Number of birds kept in a building, or on a holding on which birds have free access to more than one building, number of birds in each group of buildings on such holding</i>	<i>Number of faeces samples to be taken in the building or group of buildings on the holding</i>
1- 24	A number equal to the total number of birds up to a maximum of 20 birds
25- 29	20
30- 39	25
40- 49	30
50- 59	35
60- 89	40
90-199	50
200-499	55
500 or more	60

2. The samples to be taken shall comprise-

- (a) one chick box liner, up to a maximum of 10, for every 500 chicks delivered from each hatchery to any rearing premises on any day, such samples to be taken on the day of the arrival of the chicks there;
- (b) the carcasses of all chicks, up to a maximum of 60, from each hatchery, which are dead on arrival at any rearing premises, such samples to be taken on the day of the arrival of the chicks there;
- (c) a composite faeces sample taken from birds at 4 weeks of age and at two weeks prior to them entering the laying phase, the number of separate samples being taken in accordance with the table in paragraph 1 above;
- (d) in the case of birds whose eggs are hatched at a hatchery with a total incubator capacity of less than 1,000 eggs a composite faeces sample taken from birds at the time they enter the laying phase and at intervals of 2 weeks thereafter in accordance with the table in paragraph 1 above.

3. Samples referred to in subparagraph (d) above shall be taken under the supervision of an officer of the Minister at 56 day intervals.

PART II

Article 6(a)

SAMPLES TO BE TAKEN FROM FLOCKS OF DOMESTIC FOWL AT HATCHERIES FOR
BACTERIOLOGICAL TESTING

1. The samples to be taken shall comprise-

- (a) a composite sample of meconium taken from 250 chicks, one such sample being taken every seven days in the case of grandparent flocks and every 14 days in the case of parent flocks, from those chicks hatched from eggs supplied to the hatchery from any particular breeding flock (including flocks of less than 250 domestic fowl), or
- (b) samples comprising-
 - (i) the carcasses of all chicks which are dead in the shells of eggs supplied to the hatchery from any particular breeding flock, and
 - (ii) the carcasses of all chicks hatched from eggs supplied to the hatchery from any particular breeding flock and which have been culled.
 such samples being taken from grandparent flocks every 7 days and from parent flocks every 14 days, up to a maximum of 50 in total.

2. Samples taken for the purposes of paragraph 1 above shall be taken, every 28 days for grandparent flocks and every 56 days for parent flocks, under the supervision of an officer of the Minister.

BACTERIOLOGICAL METHODS FOR TESTING FOR SALMONELLA

1. Bacteriological method (Rappaports) for the detection of salmonella in chick box liners, composite faeces samples, meconium samples and carcasses.

Samples submitted for testing for the presence of salmonella shall be examined in the following prescribed manner on consecutive days and, where a laboratory at which samples have been received for testing on any day is unable to commence such an examination on that day, the samples shall be stored in a refrigerator at between 1°C and 4°C until required for examination.

Day 1

- (a) Chick box liners: a one gram portion shall be taken from a soiled area on each liner and the portions from separate liners shall be bulked together and placed in Buffered Peptone Water (BPW)(a), at the rate of 1 gram of liner in 10 ml of BPW up to a maximum of 10 grams in 100 ml of BPW.
- (b) Composite faeces and meconium samples: the samples shall be thoroughly mixed and a sub-sample weighing not more than 10 grams shall be placed in BPW at the rate of 1 gm sample to 10 ml BPW to a maximum of 10 grams in 100 ml BPW.
- (c) Carcasses of chicks: there shall be removed samples of the yolk sac, liver and terminal intestines to include portions of small intestines, large intestine and caecal tonsil. The samples of organs taken from the carcasses of chicks submitted shall then be bulked together and placed in BPW at the rate of 1 gram of bulked tissue in 10 ml BPW up to a maximum of 10 grams of tissue in 100 ml BPW.

The inoculated BPW shall then be incubated at 37°C for 18-24 hours.

Day 2

0.1 ml from the incubated BPW shall be inoculated into 10 ml of Rappaports Vassiliadis (RV) broth or Rappaports Vassiliadis Soya Peptone (RVS)(b) broth and incubated at $41.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 18-24 hours.

Day 3

The RV or RVS broth shall be plated out on to two plates of Brilliant Green Agar (BGA)(c) using a 10 microlitres loop. The BGA plates shall be inoculated with a droplet taken from the edge of the surface of the fluid and drawing the loop over the whole of one plate in a zigzag pattern and continuing to the second plate without recharging the loop. The space between the loop streaks shall be 0.5-1.0 cm. The plates shall be incubated at 37°C for 18-24 hours, and the RV or RVS broth reincubated at $41.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for a further 18-24 hours.

Day 4

- (i) The plates of BGA shall be examined and a minimum of 3 colonies from the plates showing suspicion of salmonella growth shall be subcultured on to a blood agar plate and a MacConkey agar plate and into biochemical composite media or equivalent. These media shall be incubated at 37°C for 18-24 hours.
- (ii) The reincubated RV or RVS broth shall be plated out, and the plates incubated, as described in Day 3.

Day 5

- (i) The incubated plates and composite media or equivalent shall be examined and the findings recorded, discarding cultures which are obviously not salmonella. Slide serological tests shall be performed using salmonella polyvalent "O" (Groups A-S) and polyvalent "H" (phase 1 and 2) agglutinating sera on selected suspect colonies collected from the blood agar or MacConkey plates. If reactions occur with one or both sera, the colonies shall be typed to Group level by slide serology.
- (ii) The plates of BGA prepared at Day 4(ii) shall be examined and further action taken as described in Day 4(i) and Day 5(i).

2. Bacteriological method (Selenite) for the detection of salmonella in chick box liners, composite faeces samples, meconium samples and carcasses.

Samples submitted for testing for the presence of salmonella shall be examined in the following

prescribed manner on consecutive days and, where a laboratory at which samples have been received for testing on any day is unable to commence such an examination on that day, the samples shall be stored in a refrigerator at between 1°C and 4°C until required for examination.

Day 1

- (a) Chick box liners: a one gram portion shall be taken from a soiled area on each liner and the portions from separate liners shall be bulked together and placed in Selenite F broth (d) at the rate of 1 gram of liner to 10 ml broth up to a maximum of 10 grams of liner in 100 ml broth.
- (b) Composite faeces and meconium samples: the sample shall be thoroughly mixed and a sub-sample weighing not more than 10 grams shall be placed in Selenite F broth at the rate of 1 gram of faeces to 10 ml broth up to a maximum of 10 grams of faeces in 100 ml broth.
- (c) Carcasses of chicks: there shall be removed samples of the yolk sac, liver and terminal intestines (to include portions of small intestines, large intestine and caecal tonsil).

The samples of organs taken from the carcasses of chicks submitted shall then be bulked together and placed in Selenite F broth at the rate of 1 gram of bulked tissue in 10 ml of broth up to a maximum of 10 grams of tissue in 100 ml broth.

The inoculated Selenite F broth shall then be incubated at 37°C for 18–24 hours

Day 2

- (i) The Selenite F broth shall be plated out on to two plates of Brilliant Green Agar (BGA) using a 10 microlitres loop. The BGA plates shall be inoculated with a droplet taken from the edge of the surface of the fluid and drawing the loop over the whole of one plate in a zigzag pattern and continuing to the second plate without recharging the loop. The space between the loopstreaks shall be 0.5 cm–1.0 cm. The plates shall be incubated at 37°C for 18–24 hours.
- (ii) The Selenite F broth shall then be reincubated at 37°C for a further 18–24 hours.

Day 3

- (i) The plates of BGA shall be examined and a minimum of 3 colonies from the plates showing suspicion of salmonella growth shall be subcultured on to a blood agar plate and a MacConkey agar plate and into biochemical composite media or equivalent. These media shall be incubated at 37°C for 18–24 hours.
- (ii) The reincubated Selenite F broth shall be plated out and incubated as described in Day 2(i).

Day 4

- (i) The incubated plates and composite media or equivalent shall be examined and the findings recorded, discarding cultures which are obviously not salmonella. Slide serological tests shall be performed using salmonella polyvalent "O" (Groups A S) and polyvalent "H" (phase 1 and 2) agglutinating sera on selected suspect colonies collected from the blood agar or MacConkey plates. If reactions occur with one or both sera, the colonies shall be typed to Group level by slide serology.
 - (ii) The plates of BGA prepared at Day 3(ii) shall be examined and further action taken as described in Day 3(i) and Day 4(i).
- (a) Buffered Peptone Water—Edel and Kampelmacher (1973) (commercially available as Oxoid CM 509, Lab M46 or equivalent).
 - (b) Rappaport Vassiliadis (RV) Broth—Vassiliadis *et al.* (1976) (commercially available as Oxoid CM 669 or equivalent) or Rappaport Vassiliadis Soya Peptone (RVS) broth (Oxoid CM 866).
 - (c) Brilliant Green Agar (Modified)—Edel and Kampelmacher (1969) (commercially available as Oxoid CM 329, Lab M34 or equivalent).
 - (d) Selenite F broth—Liefson (1936) (commercially available as Oxoid CM 395 and L.121, Lab M44a and 44b or equivalent).
- (a), (b), (c) and (d) should be reconstituted according to the manufacturer's instructions and in the case of (c) poured into 9 cm diameter plates.

References for the above substances are found in

Liefson E. (1936) *American Journal of Hygiene* 24, 423–432.

Edel, W. & Kampelmacher, E.H. (1969) *Bulletin of the World Organisation* 41, 297–306.

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SCHEDULE 3

Article 9(a)

PART I

RECORDS OF SAMPLES TAKEN IN RESPECT OF A BREEDING FLOCK OR A HATCHERY

A person whose name is entered in the Breeding Flocks Register in respect of any holding on which a breeding flock of domestic fowl is kept by him or whose name is entered in the Hatcheries Register in respect of any premises used by him as a hatchery of domestic fowl shall keep a record of samples taken in respect of the flock or hatchery (as the case may be) containing the following information-

- (i) the date on which the sample was taken;
- (ii) a description of the type of sample taken; and
- (iii) in the case of samples taken in respect of a breeding flock, the identity of the building or group of buildings from which the samples were taken and, in the case of samples taken from a hatchery, the address of the holding from which the hatching eggs from which the samples were obtained were supplied to the hatchery.

PART II

Article 9(c)

RECORDS OF THE MOVEMENT OF POULTRY, CHICKS AND EGGS ONTO AND OFF ANY HOLDING ON WHICH A BREEDING FLOCK IS KEPT

A person whose name is entered in the Breeding Flocks Register in respect of any holding on which a breeding flock of domestic fowl is kept shall keep a record of the movement of any domestic fowl, their chicks and eggs onto and off such holding which record shall contain the following information-

- (i) the date of the movement;
- (ii) the number of domestic fowl, their chicks or eggs moved;
- (iii) the identity of the building or group of buildings in which any domestic fowl, their chicks or eggs moved onto the holding were placed or from which any domestic fowl, their chicks or eggs were moved off the holding;
- (iv) in the case of any domestic fowl, their chicks or eggs moved onto the holding, the address from which they were brought there, and
- (v) in the case of any domestic fowl, their chicks or eggs moved off the holding, the address to which they were moved.

PART III

Article 9(d)

RECORDS OF THE MOVEMENT OF EGGS ONTO AND OFF, AND OF THE MOVEMENT OF CHICKS OFF, ANY PREMISES WHICH ARE USED AS A HATCHERY

A person whose name is entered in the Hatcheries Register in respect of any premises used by him as a hatchery shall keep a record of the movement of any eggs of domestic fowl onto and off, and of the movement of any chicks of domestic fowl off, such premises, which record shall contain the following information-

- (i) the date of the movement;
- (ii) in the case of the movement of any eggs onto the premises, the address of the premises from which they were moved and the number of eggs moved;
- (iii) in the case of the movement of any eggs or chicks off the premises the address to which they were moved and the number of eggs or chicks moved.

EXPLANATORY NOTE

(This note is not part of the Order)

This Order supersedes the Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989 as amended. It simplifies the requirements for registration and testing for salmonella in breeding flocks and hatcheries. It also implements, in part, Council Directive 92/117/EEC. It provides for the registration of breeding flocks (Article 3) and hatcheries (Article 4). It also provides for the taking of samples for bacteriological testing for salmonella (Articles 5 to 7).

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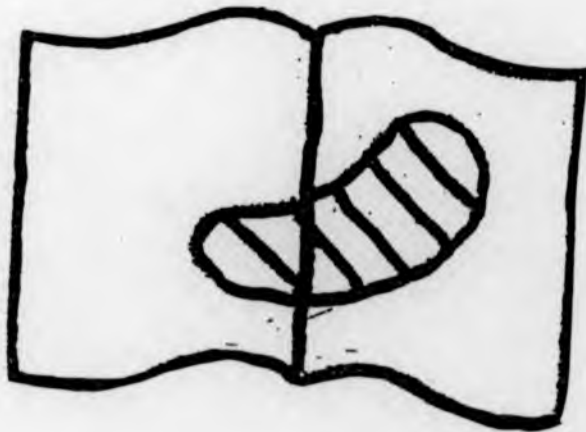
APPENDIX A.4

Salmonella in poultry investigation report (ZO4B)

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To be completed by Nominated Officer

No.	Month	Year	Serial No.

Date of visit

--	--	--	--	--

Salmonella in poultry : Investigation report

Section 1 - Details of visit

1. Name and address of farm

County		
Person in charge		Tel. No.

2. Name and address of owner of birds (for payment)

County

4. Time of (a) arrival

--

(b) departure

--

5. Persons present:

Farm

--

SVS

--

3. Name and address of veterinary surgeon

Report to be sent? YES / NO	

Section 2 - Background

4. Reason for investigation

	Tick	Comment
Traceback from food poisoning incident		
Isolation from this site: PBFHO/PLFO		
Non-statutory		
Isolation from hatchery (give name) PBFHO		
non-statutory		
Isolation from elsewhere (specify)		

7. Details of original salmonella isolate

	Poultry	Human (if known)
Sample date		
Sample type		
Lab. reference		
House(s) of origin		
Salmonella serotype/phage		
Tolworth FPI No. (if known)		

Section 3 - Summary of the site and management

(a) Species (FO=fowl, TU=turkey, DU=duck, GE=geese, OT=other (specify.))

--	--

(b) Type of fowl (L=layer, LB=layer breeder, LBGP=LBgrandparent, LBEL=LBelite, B=broiler, BB=broiler breeder, BBGP=BBgrandparent, BBEL=BBelite)

--	--	--

(c) Purpose (PR=production, RE=rearing, BR=breeding, CO=combined)

--	--

(d) Age (SA=single age, MA=multiple age)

--	--

Occupation (AA=all in / all out, CS=continuously stocked)

--	--

(e) House type (CA=cage, BA=barn, DL=deep litter, FR=free range, OT=other (specify.))

--	--

(f) Registered under Poultry Flocks Orders?

YES / NO

--

Section 4 - Details of Nominated Officer

Name and full postal address

Postcode	Telephone No.

Section 5 - Disease Security Policy

No.	Month	Year	Serial No.

Site

Is the site secured by a continuous fox-proof perimeter fence? YES NO

Are visitors always logged in a visitor book? YES NO

Do visitors wear adequate protective clothing? (at least waterproof footwear and clean overalls) YES NO

Does the site supply the visitors' protective clothing? YES NO

Are visitors/delivery persons allowed inside poultry houses? YES NO

Are there any other poultry sites/processing plants within 11km? YES NO

On entry to the site are vehicles:

cleaned? YES NO

disinfected? YES NO

Operation

Are houses effectively protected against entry by wild birds? (netted windows, vents) YES NO

Age of poultry houses on site (years) 0-1 2-3 4-5 6-10 11-20 21-30 31-40 41-50 51-60 61-70 71-80 81-90 91-100

State of repair of houses, pens and equipment

Excellent Satisfactory Poor

Evidence of mice/rats on site (tick all that apply)

Live rodents Dead rodents Droppings

Damage to building/equipment

Holes/runs No evidence

Other domestic animals on site (tick all that apply)

Cats/dogs Cattle Sheep

Pigs Other birds Other

Are disinfectant footbaths in use on site?

Yes No

If YES:

- is there one footbath outside every house? Yes No

- how often is the disinfectant changed?

Daily Weekly Less Frequently

Ventilation system in houses?

Natural Mechanical (sides) Mechanical (roof)

Is drinking water chlorinated? (i.e. mains or chlorine added to own supply) Yes No

The intercrop routine (tick all that apply)

- are houses Swept Powerwashed Steamcleaned

- are fixtures Disinfected Fogged Fumigated

- are fixtures Dismantled Steamcleaned Disinfected

- are bacteriological tests carried out? YES NO

- is manure removed from site immediately? YES NO

Personnel

Give number of people working on site, including owner/manager 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

Have hygiene rules written by management been seen and agreed by staff? YES NO

Do personnel have contact with other poultry? YES NO

Is appropriate protective clothing used (at least waterproof footwear and clean overalls)? YES NO

Is a different set of protective clothing used for each house? YES NO

What hygiene facilities are used? (Tick all that apply)

Handwash basin Changing room Shower

Birds / Eggs

Do birds come from a single supplier? YES NO

Have all suppliers' salmonella test programmes been examined before delivery? YES NO

Is delivery in vehicles which are dedicated to bird/egg delivery? YES NO

Are eggs collected more than once daily? YES NO

Are tests for salmonella undertaken in addition to those required by the Orders?

Feed

Is feed supplied from a single feedmill? YES NO

Is any feed home mixed? YES NO

Is animal protein included in any feed? YES NO

Is delivery in vehicles which are dedicated to feed delivery? YES NO

Is more than one type of feed in use? YES NO

How is feed delivered?

Into bulk hoppers In bags Combined

Is the feed treated with (tick all that apply)

pelleting? Acid? Antibiotic?

probiotic? Heat? Other additives?

Names

Feed supplier(s) 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

Destination(s) of hatching eggs (if any) 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

Section 6 - House by house information

No.	Month	Year	Serial No.

A house is a building or part of building with solid partitions and its own ventilation system. Asterisk those sharing a common plot and append a sketch plan of the site. Record further house details on continuation sheet.

	1	2	3	4
House number	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
name	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
type*	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Species*	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
type of fowl*	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
no. of females	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
no. of males	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Age (weeks)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
in lay Y/N	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Date of entry to site	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
entry to house planned	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
depopulation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
source of chicks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
hatchery name	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
flock code/s	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
delivery date	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
no. delivered	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
rearing site	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
samples taken	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
no. birds	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
no. cloacal swabs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
no. faeces/litter	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
no. dust	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
no. blood	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
other (specify type and no.)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Use codes in Section 3 on page 1

Section 7 - Investigation Officer comments

Record any medication (type/concentration/duration in 28 day period before investigation), movements of eggs/birds, mortality and source of infection if known or suspected.

Section 8 - Results of serology (CVL)/bacteriology (VIC/Lasswade)

* If applicable put 'S' in column to indicate which batches are cloacal swabs.
 † If positive record unique sequential number and serogroup, record negative as neg.

No.	Month	Year	Serial No.

Lab. reference

House	ELISA	Batch			Culture Result †		House	ELISA	Batch			Culture Result †			
		S*	Letter	Size	Liver/Gonad	Intestine or Swab*			S*	Letter	Size	Liver/Gonad	Intestine or Swab*		
1							2								
3							4								

Section 9

Preliminary report (serology/serogroup) Final report (serology/serotype)

Signed by	Preliminary Report		Final Report	
	Name	Date	Name	Date
Investigation Officer				
Nominated Officer				

Distribution of report copies	Date Sent		Date Sent	
	Fax	Paper	Fax	Paper
SVO (salmonella in poultry) Tolworth or Pentland House				
DVO				
Supt VIO				
Owner				
Epidemiology Unit CVL				

Section 6(A) - House by house information

A house is a building or part of building with solid partitions and its own ventilation system. Asterisk those sharing a common plot and append a sketch plan of the site. Record further house details on continuation sheet.

No.	Month	Year	Serial No.

	5	6	7	8
house number	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
name	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
type*	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
species*	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
type of fowl*	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
n. of females	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
n. of males	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
age (weeks)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
in lay Y/N	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
date of entry to site	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
entry to house	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
planned depopulation	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
source of chicks hatchery name	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
flock code/s	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
delivery date	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
no. delivered	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
rearing site	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
samples taken	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
no. birds	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
no. cloacal swabs	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
no. faeces/fitter	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
no. dust	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
no. blood	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
other (specify type and no.)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

* codes in Section 3 on page 1

Section 7(A) - Investigation Officer comments

Record any medication (type/concentration/duration in 28 day period before investigation), treatments of eggs/birds, mortality and source of infection if known or suspected.

Section 8(A) – Results of serology (CVL)/ bacteriology (VIC/Lasswade)

* If applicable put 'S' in column to indicate which batches are cloacal swabs.
 † If positive record unique sequential number and serogroup, record negative as neg.

No.	Month	Year	Serial No.

Lab. reference

House	ELISA	Batch			Culture Result †		House	ELISA	Batch			Culture Result †	
		S*	Letter	Size	Liver/ Gonad	Intestine or Swab*			S*	Letter	Size	Liver/ Gonad	Intestine or Swab*
5							6						
7							8						

Section 9(A)

Preliminary report (serology/serogroup) Final report (serology/serotype)

Signed by	Preliminary Report		Final Report	
	Name	Date	Name	Date
Investigation Officer				
Nominated Officer				

Distribution of report copies	Date Sent		Date Sent	
	Fax	Paper	Fax	Paper
SVO (salmonella in poultry) Tolworth or Pentland House				
DVO				
Supt VIO				
Owner				
Epidemiology Unit CVL				

APPENDIX A.5

Guidelines for the retrospective completion of form ZO4B

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SALMONELLA IN POULTRY CASE CONTROL STUDY

GUIDELINES FOR THE RETROSPECTIVE COMPLETION OF FORM ZO4B

The CVL is conducting a study to investigate the risk factors associated with poultry flock infection with *S. enteritidis*. Initially only breeder flocks are to be considered. The study will involve the analysis of data obtained from the revised Zoonosis Order visit report form which was introduced in 1993. However, in order to increase the number of flocks in the analysis, flocks investigated during 1992 are also to be included where possible. To utilise these flock investigations in the study, we require some information about the sites additional to that given at the time of the field investigation. The following guidelines explain how to complete a retrospective ZO4B for flocks investigated during 1992 and thus provide the required missing information for the CVL study.

A partially completed new style ZO4B has been prepared for each investigation visit carried out during 1992 that is eligible for inclusion in the study.

On receipt of a partially completed ZO4B:-

1. Check all details correspond with your records. Notify Mrs S. Evans, CVL, of any discrepancies.
2. Use the draft letter attached (salmonella in poultry field investigation reports for 1992) to approach the flock owner. Enclose a copy of the partially completed ZO4 with the letter.
3. Contact the flock owner to discuss the completion of the form 7-10 days after sending the introductory letter. Notify Mrs S. Evans if the owner is unwilling to provide the data.
4. If the owner agrees to co-operate, he or she should then be approached by letter, telephone or a further visit as appropriate to provide the missing information on the ZO4.

To complete a ZO4B for a 1992 flock investigation:-

5. Check with the flock owner that the details already on the form are correct. Make a note of any discrepancies and attach to the form.
6. Complete Section 5 (Disease Security Policy) with the owner. As far as possible, this should be completed retrospectively ie. the answers given should refer to disease security policy at the time of the investigation. Please state if any information is not known.
7. You are not required to complete any sections with a diagonal line ruled through them.
8. Complete any additional missing information which has been highlighted on the form.
9. Carefully check the form after completion and then return to Mrs S. Evans, CVL.

Return all forms and direct queries to:-

**Mrs S. Evans MRCVS, Epidemiology Department, Central Veterinary Laboratory,
New Haw, Addlestone, Surrey KT15 3NB.**

Tel: 0932 341111 ext 2459

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Original

APPENDIX A.6

Introductory letter to owners of flocks with 1992 investigations

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In
Original

(MAFF headed letter paper)

(Flock owner:
Name
Address)

(Date)

Dear (flock owner)

SALMONELLA IN POULTRY FIELD INVESTIGATION REPORTS FOR 1992

I am writing to ask for your co-operation in a research project which is being conducted by the Epidemiology Department of the Central Veterinary Laboratory. The study aims to investigate the importance of various factors associated with risk of poultry flock infection with salmonella. The results will benefit both the poultry industry and vets in developing effective measures to control salmonella infection in poultry.

A revised visit report form was introduced in 1993 to gather information on management factors and disease security at all statutory flock investigations. This data will be examined by the study. However, in order to increase the number of flocks in the analysis, flocks investigated during 1992 will be included on a historical basis. The form completed at these 1992 visits was less detailed than the current version. Therefore, to include these flocks in the study, it is necessary to obtain some information about the site additional to that given at the time of the investigation.

From our current data, a partially completed new style visit report form has been prepared for an investigation carried out at your premises last year. A copy of the form is enclosed with this letter for your consideration. It will be of great value for the purpose of the study if the supplementary information on the questionnaire is obtained. The required information refers to the flock at the time of the original visit so you may need to consult your records in order to complete the form.

I hope that you will be able to spare the time to provide this additional information. All details will be treated in confidence and summarised in an anonymous format for the analysis and participation is entirely voluntary.

I will contact you shortly to arrange for the completion of the rest of the form if you are in agreement. In the mean time, please contact me if you have any queries.

Yours sincerely,

(Your name)

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APPENDIX A.7

Guidelines for the completion of a poultry breeder flock management questionnaire

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SALMONELLA IN POULTRY CASE CONTROL STUDY
GUIDELINES FOR THE COMPLETION OF A POULTRY BREEDER FLOCK
MANAGEMENT QUESTIONNAIRE

The CVL is conducting a study to investigate the risk factors associated with poultry flock infection with *S. enteritidis*. Initially only breeder flocks are to be considered. The study will involve the analysis of data obtained from the revised Zoonosis Order visit report form which was introduced in 1993. However, in order to increase the ability of this study to detect factors associated with salmonella infection it is necessary to make comparisons with non-investigated "control" flocks. Although these flocks might not be truly negative, they are subject to the same monitoring process as "cases" and, therefore, can be considered as "controls" for the purposes of this study. These guidelines explain how to complete a Poultry Breeder Flock Management Questionnaire (PBFMQ) for a "control" flock.

A partially completed PBFMQ has been prepared for each randomly selected "control" flock eligible for inclusion in the study.

On receipt of a partially completed PBFMQ:-

1. Check details correspond with your records. The flock should not have recently been subject to a salmonella investigation. Notify Mrs S. Evans, CVL, of any discrepancies.
2. Use the draft letter attached (salmonella in poultry case control study) to approach the flock owner. Enclose a copy of the PBFMQ.
3. Contact the flock owner to discuss the completion of the form 7-10 days after sending the introductory letter. Notify Mrs S. Evans if the owner is unwilling to participate in the study.
4. If the owner agrees to co-operate, he or she should then be approached by letter, telephone or visit to complete the PBFMQ. In most instances it will be necessary to visit the premises to complete the questionnaire. The PBFMQ should be completed as soon as possible after contacting the flock owner to avoid biasing the age distribution of birds in "control" flocks but the visit should not take place during the intercrop (empty) period.

To complete a Poultry Breeder Flock Management Questionnaire:-

5. Check with the flock owner that the details already on the form are correct. Make a note of any discrepancies and attach to the form.
6. Complete the questionnaire by interviewing the flock owner. As far as possible, the PBFMQ should be completed in the same way as a ZO4B (on which the form is based). Complete the form for the current flock of birds but particular care should be taken to complete section 5 if there have been any recent changes in disease security policy or management at the site. All changes should be noted together with the dates that they were effective. Attach a separate piece of paper if necessary.
7. You are not required to obtain any samples for bacteriological examination.
8. Carefully check the form after completion and then return to Mrs S. Evans, CVL.

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APPENDIX A.8

Introductory letter for control flock owners

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In
Original

(MAFF headed letter paper)

(Flock owner:
Name
Address)

(Date)

Dear (flock owner)

SALMONELLA IN POULTRY CASE CONTROL STUDY

I am writing to ask for your co-operation in a research project which is being conducted by the Epidemiology Department of the Central Veterinary Laboratory. The study aims to investigate the importance of various factors associated with risk of poultry flock infection with salmonella. The results will benefit both the poultry industry and vets in developing effective measures to control salmonella infection in poultry.

A questionnaire is completed at all statutory salmonella flock investigations to gather information on management factors and disease security at the site. These data are to be examined by the study. However, in order to make comparisons between infected and non-infected flocks it is necessary to complete a similar questionnaire in a random sample of "control" flocks which have not been subject to a statutory investigation. Analysis of the data will then highlight any differences between the two groups of flocks that may be associated with an increased or decreased risk of salmonella infection.

Your site has been randomly selected to act as one of the "control" flocks for this study. Participation in the study involves the completion of a short questionnaire with my assistance. There is no requirement to provide any samples for bacteriological examination. I have enclosed the study questionnaire for your consideration.

I hope that you will be able to spare the time to provide this information. Without the co-operation of flock owners, such as yourself, the study will be of limited value as the collection of "control" information from flocks which have not been subject to a salmonella investigation is vital for the analysis. All details will be treated in confidence and summarised in an anonymous format for the analysis. Participation is entirely voluntary.

I will contact you shortly to arrange for the completion of the form if you are in agreement. In the mean time, please contact me if you have any queries.

Yours sincerely,

(Your name)

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APPENDIX A.9

Poultry breeder flock management questionnaire

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Poultry Breeder Flock Management Questionnaire

To be completed by the VIO For each randomly selected "control" breeding flock willing to participate in the study.

Section 1 - Identifying Information

1. Name and address of registered person _____

2. Name and address of premises (if different) _____

3. PBFHO Registration number _____
4. Name of VIO _____
5. Name of VI Centre _____
6. Date of visit _____ 19 ____

Section 2 - Flock Summary

7. Type of fowl (LB=layer breeder, LBGP=LB grandparent, LBEL=LB elite, BB=broiler breeder, BBGP=BB grandparent, BBEL=BB elite) _____
8. Age (SA=single age, MA= multiple age) _____
9. Occupation (AA=all in/ all out, CS=continuously stocked) _____
10. Please specify any other type(s) of poultry on the premises (tick boxes that apply)
None Layer Broiler Hatchery
Other (please specify) _____

Please complete Section 3 (Disease Security Policy), Section 4 (House by house information) and Section 5 (Management changes) then return this complete form to:-

Mrs SJ Evans MRCVS Epidemiology Department, Central Veterinary Laboratory,
New Haw, Addlestone, Surrey KT15 3NB

to whom any queries should also be addressed (Tel: 0932 341111 ext. 2459)

Section 3 - Disease Security Policy

TICK boxes that apply. State if answer UNKNOWN.

		YES	NO	COMMENTS
Site				
Is the site secured by a continuous fox-proof perimeter fence?		<input type="checkbox"/>	<input type="checkbox"/>	
Are visitors always logged in a visitor book?		<input type="checkbox"/>	<input type="checkbox"/>	
Do visitors wear adequate protective clothing? (at least waterproof footwear and clean overalls)		<input type="checkbox"/>	<input type="checkbox"/>	
Does the site supply the visitors' protective clothing?		<input type="checkbox"/>	<input type="checkbox"/>	
Are visitors/delivery persons allowed inside poultry houses?		<input type="checkbox"/>	<input type="checkbox"/>	
Are there any other poultry sites/processing plants within 1km?		<input type="checkbox"/>	<input type="checkbox"/>	
On entry to the site are vehicles:				
	cleaned?	<input type="checkbox"/>	<input type="checkbox"/>	
	disinfected?	<input type="checkbox"/>	<input type="checkbox"/>	
Operation				
Are houses effectively protected against entry by wild birds? (netted windows, vents)		<input type="checkbox"/>	<input type="checkbox"/>	
Age of poultry houses on site (years)		<input type="checkbox"/>		
State of repair of houses, pens and equipment	Excellent	<input type="checkbox"/>		Satisfactory <input type="checkbox"/> Poor <input type="checkbox"/>
Evidence of mice/rats on site (tick all that apply)	Live rodents	<input type="checkbox"/>		Dead rodents <input type="checkbox"/> Droppings <input type="checkbox"/>
	Damage to building/equipment	<input type="checkbox"/>		Holes/runs <input type="checkbox"/> No evidence <input type="checkbox"/>
Other domestic animals on site (tick all that apply)	Cats/dogs	<input type="checkbox"/>		Cattle <input type="checkbox"/> Sheep <input type="checkbox"/>
	Pigs	<input type="checkbox"/>		Other birds <input type="checkbox"/> Other <input type="checkbox"/>
Are disinfectant footbaths in use on site?	Yes	<input type="checkbox"/>		No <input type="checkbox"/>
If YES:				
- is there one footbath outside every house?	Yes	<input type="checkbox"/>		No <input type="checkbox"/>
- how often is the disinfectant changed?	Daily	<input type="checkbox"/>		Weekly <input type="checkbox"/> Less Frequently <input type="checkbox"/>
Ventilation system in houses?	Natural	<input type="checkbox"/>		Mechanical (sides) <input type="checkbox"/> Mechanical (roof) <input type="checkbox"/>
Is drinking water chlorinated? (i.e. mains or chlorine added to own supply)	Yes	<input type="checkbox"/>		No <input type="checkbox"/>
The intercrop routine (tick all that apply)				
- are houses	Swept	<input type="checkbox"/>		Powerwashed <input type="checkbox"/> Steamcleaned <input type="checkbox"/>
- are fixtures	Disinfected	<input type="checkbox"/>		Fogged <input type="checkbox"/> Fumigated <input type="checkbox"/>
- are bacteriological tests carried out?	Dismantled	<input type="checkbox"/>		Steamcleaned <input type="checkbox"/> Disinfected <input type="checkbox"/>
- is manure removed from site immediately?		<input type="checkbox"/>	<input type="checkbox"/>	
Personnel				
Give number of people working on site, including owner/manager		<input type="checkbox"/>		
Have hygiene rules written by management been seen and agreed by staff?		<input type="checkbox"/>	<input type="checkbox"/>	
Do personnel have contact with other poultry?		<input type="checkbox"/>	<input type="checkbox"/>	
Is appropriate protective clothing used (at least waterproof footwear and clean overalls)?		<input type="checkbox"/>	<input type="checkbox"/>	
Is a different set of protective clothing used for each house?		<input type="checkbox"/>	<input type="checkbox"/>	
What hygiene facilities are used? (Tick all that apply)	Handwash basin	<input type="checkbox"/>		Changing room <input type="checkbox"/> Shower <input type="checkbox"/>
Birds / Eggs				
Do birds come from a single supplier?		<input type="checkbox"/>	<input type="checkbox"/>	
Have all suppliers' salmonella test programmes been examined before delivery?		<input type="checkbox"/>	<input type="checkbox"/>	
Is delivery in vehicles which are dedicated to bird/egg delivery?		<input type="checkbox"/>	<input type="checkbox"/>	
Are eggs collected more than once daily?		<input type="checkbox"/>	<input type="checkbox"/>	
Are tests for salmonella undertaken in addition to those required by the Orders?		<input type="checkbox"/>	<input type="checkbox"/>	
Feed				
Is feed supplied from a single leadmill?		<input type="checkbox"/>	<input type="checkbox"/>	
Is any feed home mixed?		<input type="checkbox"/>	<input type="checkbox"/>	
Is animal protein included in any feed?		<input type="checkbox"/>	<input type="checkbox"/>	
Is delivery in vehicles which are dedicated to feed delivery?		<input type="checkbox"/>	<input type="checkbox"/>	
Is more than one type of feed in use?		<input type="checkbox"/>	<input type="checkbox"/>	
How is feed delivered?	Into bulk hoppers	<input type="checkbox"/>		In bags <input type="checkbox"/> Combined <input type="checkbox"/>
Is the feed treated with (tick all that apply)	pelleting?	<input type="checkbox"/>		Acid? <input type="checkbox"/> Antibiotic? <input type="checkbox"/>
	probiotic?	<input type="checkbox"/>		Meal? <input type="checkbox"/> Other additives? <input type="checkbox"/>
Names				
Feed supplier(s)				
Destination(s) of hatching eggs (if any)				

Section 4 - House by house information

A house is a building or part of a building with solid partitions and its own ventilation system. Record further house details on continuation sheet.

	1	2	3	4
House name/no.				
No. females				
No. males				
Age (weeks)				
In lay (Yes/No)				
Date of entry to site				
entry to house				
planned depopulation				
Source of chicks hatchery name				
flock code/s				
delivery date				
no. delivered				
Rearing site				

Section 5 - Management changes

Record any changes in management of the site since 1 January 1992 (by referring to the questions in section 3, for example, source of birds, feed supply, destination of hatching eggs, improvements to buildings or changes in hygiene rules). Give dates, if appropriate.

Section 4 - House by house information (continuation sheet)

	5	6	7	8
House name/no.				
No. females				
No. males				
Age (weeks)				
In lay (Yes/No)				
Date of entry to site				
entry to house				
planned depop.				
Source of chicks hatchery name				
flock code/s				
delivery date				
no. delivered				
Rearing site				
	9	10	11	12
House name/no.				
No. females				
No. males				
Age (weeks)				
In lay (Yes/No)				
Date of entry to site				
entry to house				
planned depop.				
Source of chicks hatchery name				
flock code/s				
delivery date				
no. delivered				
Rearing site				

APPENDIX B

A cross-sectional survey of thermophilic campylobacter infection of broiler flocks in England and Wales

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APPENDIX B.1

Farm sampling protocol

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CVL Campylobacter Broiler Study - Farm Sampling Protocol

The sample kit provided should contain the following items:-

- a) Farm Questionnaire and reply-paid envelope for sending questionnaire to CVL
- b) 16 swabs in a mini-grip plastic bag
- c) Labelled empty mini-grip plastic bag
- d) Reply-paid jiffy bag for sending swabs to Q Laboratories Ltd.
- e) Sealed brown envelope (for selecting the house to be swabbed)

To select the house of birds to be swabbed:

1. 16 birds that are at least 35 days old are to be swabbed from one house on the farm. A house is a building or part of a building with solid partitions and its own ventilation system. On the front of the sealed brown envelope, list the name/number of all broiler houses which contain birds which are at least 35 days old today. Do not list empty houses or those with birds less than 35 days old.
2. You will see that each house you have listed has been given a code letter. Now, open the envelope and you will find a piece of paper with a line of letters written on it. The house which you should select is the one which corresponds to the first possible code letter seen when reading from left to right eg. if you have listed 3 houses (coded "A", "B" and "C") and you open the envelope to find a random selection of letters as follows: N F I D C P E O B A G K J H M L the house of birds to be swabbed is the one on the front of the envelope which has been given the code letter "C" as this is the first letter of the three possible letters that is seen when reading from left to right.

To obtain cloacal swabs from birds in the selected house:

1. Write the identifying information (including the selected house name or number) on the label of the empty plastic bag. Take the bag of swabs and the labelled plastic bag into the selected house.
2. Individual cloacal swabs should be taken from 16 birds in the selected house. 4 birds should be chosen from different areas in each quarter of the house so that a total of 16 birds are swabbed.
3. If possible, get someone to hold the birds for you to swab.
4. To obtain a cloacal swab, remove a sterile swab from its wrapping and insert the tip gently into the cloaca. Rotate in both directions and gently remove. Remove the cap from the tube of medium and place the swab into the medium pushing the swabs' cap firmly into the tube. Put all 16 cloacal swabs into the labelled mini-grip plastic bag and seal the bag. Place left-over wrapping and caps in the unlabelled plastic bag and dispose of in a rubbish bin on the farm.

To complete the farm questionnaire:

The questionnaire should be completed by interviewing the owner or manager of the site and following the written instructions given. It is important that once completed, it is read through again to check for mistakes and to check that all the questions have been answered.

Postage instructions:

1. Place the sealed, clearly labelled, mini-grip plastic bag containing the cloacal swabs into the jiffy bag and seal securely. Write your name and the farm address on the back of the jiffy bag and post immediately to Q Laboratories Ltd. No postage stamps are required.
2. Place the completed farm questionnaire together with the brown envelope and piece of paper used for selecting the house that was swabbed into the envelope addressed to Mrs Evans at the Central Veterinary Laboratory. Post to Mrs Evans as soon as possible. No postage stamps are required.

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APPENDIX B.2

Survey questionnaire

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CAMPYLOBACTER BROILER SURVEY

Mrs S J Evans MRCVS,
Epidemiology Department, Central Veterinary Laboratory,
New Haw, ADDLESTONE, Surrey KT15 3NB
Tel: 0932 341111 Ext. 2459

Office Use Only

Tick all boxes that apply and give written details using CAPITAL LETTERS

SECTION 1 : Identifying Information

Name and address of farm _____

Post Code _____

Telephone No. (incl. code)

Type of farm : Broiler Company managed Contract (privately owned)

Name and address of Processing Plant _____

Post Code _____

Distance from farm to Processing Plant _____ miles

Details of person completing the questionnaire:

Your name _____

Your position : Owner Manager Area Farms Manager
(Broiler Company)

Other (specify) _____ Today's date _____

SECTION 2 : Summary of Site and Management (tick ALL that apply)

1. Apart from the broiler flock, are there any other poultry on the farm?

No Yes: Fowl: Layers Brooders

Other species: Turkeys Ducks

2. Are there any other species of domestic animals on the farm?

No Yes: Cattle Sheep Pig

Horse Dog Cat

Other (specify) _____

Is the WHOLE site emptied between crops i.e. an all in/all out system?

No Yes

Office Use Only

1

2

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SECTION 3 : Disease Security at the Site

1. Is the site secured by a continuous perimeter fence? NO YES
2. Is there MORE THAN ONE entrance to the site? NO YES
3. Is there a warning notice at the site entrance? NO YES
4. Do vehicles drive through a disinfectant wheel bath on entry? NO YES
5. How far is it to the nearest neighbouring farm with poultry? _____ miles
6. Please give the number of personnel working at the site:
 a) Full time _____ b) Part time _____
7. Since the current crop of chicks were placed (delivered) : NO YES
 a) have any personnel helped at other poultry sites? NO YES
 b) have any workers from other poultry sites helped at this site? NO YES

8. Indicate which visitors to the site carry out the following hygienic precautions:

For each box place a TICK IF YES or a CROSS IF NO					For each visitor, give the number of visits to the site since chick delivery
Precaution	a) Disinfect Vehicles	b) Wear Clean Overalls	c) Disinfect Footwear	d) Enter Poultry Houses	
Visitor					
Hatchery Staff					
Feed delivery					
Maintenance					
Dead bird removal					
Pest Contractor					
Vet					
Area Manager (Broiler Company)					
Catchers					
Cleaners					
Other visitors					

9. Is there a written hygiene protocol for personnel working at the site? NO YES
10. What hygiene facilities are used by personnel?
 None Wash basin Shower NO YES
11. Do any personnel keep poultry, pigeons or pet birds at home? NO YES
12. Do any livestock graze on the site? NO YES
13. Do foxes get into the site? NO YES

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SECTION 4 : House by House Information (for the current crop)

Give details of ALL broiler houses on the site

- * A house is a building or part of a building with solid partitions and its own ventilation system.
 † Give the adjacent house name or number ONLY when there is more than one "house" per building.

	1	2	3	4
House* name or number				
Occupied (Yes / No)				
Number of chicks placed or CAPACITY if empty				
Age of birds today (days)				
Source of birds:				
Hatchery Name				
Breed of birds				
Parent flock code(s)				
Adjacent house name or number †				

	5	6	7	8
House* name or number				
Occupied (Yes / No)				
Number of chicks placed or CAPACITY if empty				
Age of birds today (days)				
Source of birds:				
Hatchery Name				
Breed of birds				
Parent flock code(s)				
Adjacent house name or number †				

Continue on another sheet if necessary

Name and address of farm _____

 _____ Post Code _____

SECTION 4 : House by House Information (continuation sheet)

* A house is a building or part of a building with solid partitions and its own ventilation system.

† Give the adjacent house name or number ONLY when there is more than one "house" per building.

	9	10	11	12
House* name or number				
Occupied (Yes / No)				
Number of chicks placed or CAPACITY if empty				
Age of birds today (days)				
Source of birds: Hatchery Name				
Breed of birds				
Parent flock code(s)				
Adjacent house name or number [†]				

	13	14	15	16
House* name or number				
Occupied (Yes / No)				
Number of chicks placed or CAPACITY if empty				
Age of birds today (days)				
Source of birds: Hatchery Name				
Breed of birds				
Parent flock code(s)				
Adjacent house name or number [†]				

Mrs S J Evans MRCVS
Epidemiology Department, Central Veterinary Laboratory
New Haw, ADDLESTONE, Surrey KT15 3NB
Tel: 0932 341111 Ext. 2459

SECTION 5 : Sampled House Information

Cloacal swabs should be taken from 16 birds in one randomly selected house on the site following the sampling protocol supplied. The next questions refer to this selected house only.

House name or number _____

1. Type of building : Controlled ventilation broiler shed

Other (specify) _____

2. Age of building (years) _____

3. Size of house : Length : _____ feet Width : _____ feet

4. Floor construction : Concrete Rammed chalk Earth Other

5. Wall construction : Concrete block (full height) Brick Other

Boarding on concrete block Timber

6. Ventilation System : Natural Roof fans Side fans Ducted

7. Source of birds water : Mains Borehole Well River

8. Is chlorine or similar added to the water source? No Yes

9. Is a water sanitizer added to the header tanks? No Yes

10. Water drinkers : Cup Nipple

Bell Give diameter of bell : _____ inches

11. Feeding system : Hopper Pan Chain Other

12. Are wild birds seen inside the house? No Yes

13. Is the house or equipment due for repairs? None Minor Major

14. Is the house surrounded by a concrete apron? No Ends only Yes

15. Litter type : Wood shavings Chopped straw Shredded paper

Other (specify) _____

16. Litter condition at present : Good Caked in places Wet

17. Was any litter re-used from the last crop? : No Yes

18. Manure disposal : Spread on the farm Removed immediately from the site

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SECTION 5 : (continued)

19. What protective clothing is worn by personnel?

Waterproof footwear Overalls Gloves Hat Mask

20. Is there a disinfectant boot dip outside the house?

No Yes : In food store Yes : Outside building

21. Give the name of the disinfectant in the boot dip : _____

22. How frequently is the disinfectant solution in the boot dip changed?

Once per crop Fortnightly or weekly Every few days

23. Is an alcohol rinse or bactericidal soap used by personnel when hand washing?

No Yes

24. Dead bird disposal : On site : Burial Composted Incinerated

Off site : Removed by a Contractor Knackers

25. When the house was cleaned after the last crop, was the house?

Vacuumed Blown Power washed

Steam cleaned Fogged Fumigated *

* Generation of formaldehyde vapour from heated paraformaldehyde or formalin and potassium permanganate

26. Give the name of the disinfectant/s used when the house was last cleaned :

1. _____ 2. _____

27. How long was the house empty after the new litter was put in? _____ days

28. Are rodents currently present on the site? No Yes: Rats Yes: Mice

29. How frequently are mice or rats seen (live or dead)?

Daily Weekly Less frequently

30. Is a rodent control programme operated?

No: go to q.32 Yes : By Site Yes : By a Pest Contractor

31. Please give the name of the rodenticide (bait) or other control product used:

32. What is the level of litter beetle infestation in the house?

Heavy Moderate Light

33. Is an insecticide (beetle control product) used during clean out?

No Yes : Every few crops Yes : Every crop

ID	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
43	<input type="checkbox"/>		
44	<input type="checkbox"/>		
45	<input type="checkbox"/>		
46	<input type="checkbox"/>		
47	<input type="checkbox"/>		
48	<input type="checkbox"/>		
49	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
50	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
51	<input type="checkbox"/>		
52	<input type="checkbox"/>		
53	<input type="checkbox"/>		
54	<input type="checkbox"/>		
55	<input type="checkbox"/>		
56	<input type="checkbox"/>		
57	<input type="checkbox"/>		
58	<input type="checkbox"/>		

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APPENDIX C

A longitudinal study of thermophilic campylobacter infection of poultry broiler flocks in Great Britain

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APPENDIX C.1

Slaughter sampling instructions (previous flock)

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CVL CAMPYLOBACTER BROILER STUDY

PREVIOUS FLOCK SLAUGHTER SAMPLING AT FINAL DEPOPULATION OF BROILER HOUSE

Instructions

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- 1 Please sample 16 birds from the last batch of birds to be slaughtered from one broiler house (final depopulation) on each broiler site involved in the survey.
- 2 Cloacal swabs should be taken from the birds immediately prior to slaughter at the processing plant eg. at shackling or, if more convenient, sampling can be conducted at the farm on the day or day before slaughter.
- 3 Great care should be taken to obtain cloacal swabs without the swab touching the feathering of the bird or any equipment in order to avoid cross-contamination when samples are taken at the processing plant.
- 4 Please ensure that the broiler house of origin of the birds is correctly recorded as it will be birds in the next production cycle housed in this same broiler house which will be studied. This is referred to on this form as the study broiler house (S).
- 5 Complete this form and the label on the plastic bag containing the swabs and send with the swabs immediately to the CVL in the reply-paid packaging provided.

Identifying information

Name of farm _____

Farm address _____

Name of farm owner / manager _____ Tel : _____

Broiler company _____ Processing plant _____

Sampling information

Name or number of broiler house of origin of birds (S) _____

Age of birds today (days) _____ Date of sampling _____

Place of sampling: Processing plant Farm (tick one box)

Date of current crop final depopulation : (1) house (S) _____ (2) site _____

Expected date of next chick placement : (1) house (S) _____ (2) site _____

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APPENDIX C.2

Post-cleansing and disinfection sampling instructions

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CVL CAMPYLOBACTER BROILER STUDY

INSTRUCTIONS FOR OBTAINING ENVIRONMENTAL SAMPLES FROM BROILER HOUSES POST CLEANSING AND DISINFECTION

Name of farm _____ Broiler house _____
ID

- 1 Post-C&D environmental sampling should be conducted in the study broiler house before chick placement. Write identifying information on label of empty plastic bag.
- 2 Immediately prior to sampling, swabs should be moistened by dipping into sterile saline (supplied) and gently squeezing against the inside of the container in order to remove excess moisture.
- 3 Sampling should be conducted as directed collecting swabs from each of the following sample sites. In all cases swab as large an area as possible so that swabs are heavily contaminated. Clearly label the sample site or swab number on each swab.
 - 3.1 Header Tank
Swabs 1 & 2 Swab moist scum at bottom of empty tank and water level mark on side of tank (if the tank is not empty, swab water level mark).
 - 3.2 Drinkers
Swabs 3 & 4 Swab water reservoir of two bell or cup drinkers per swab (if nipple drinkers, unscrew nipple carefully and swab inside water line).
 - 3.3 Wooden Support Posts (supporting roof in middle of broiler house)
Swabs 5 & 6 Swab from the base of posts preferably in cracked or damaged wood.
 - 3.4 Slave Feed Hoppers
Swab 7 Swab from any residual feed particles in bottom of hopper,
Swab 8 Swab at the point of entry into the chain feeder system.
 - 3.5 Walls
Swabs 9 & 10 Swab from beneath flaps of asphalt over brick or concrete stub walls (if no flaps, swab cracks and holes in cladding where litter may be trapped).
 - 3.6 Floor
Swabs 11 & 12 Swab from cracks or expansion joints in the floor,
Swabs 13 & 14 Swab in corners of house (stick swab in as deeply as possible),
Swabs 15 & 16 Swab from a damp open floor surface
- 4 Put all 16 labelled environmental swabs in the labelled plastic bag and seal the bag. Place left over wrapping and caps and all packaging for the saline in the other plastic bag and suitably dispose.
- 5 Place the sealed plastic bag of swabs in the return jiffy bag and seal securely.
- 6 Write the farm address on the back of the jiffy bag and post the same day to CVL.
- 7 Now please complete the Cleansing and Disinfection Questionnaire and return separately to CVL in the reply paid envelope attached.

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APPENDIX C.3

Instructions for live bird sampling for campylobacter

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CVL CAMPYLOBACTER BROILER STUDY

INSTRUCTIONS FOR LIVE BIRD SAMPLING FOR CAMPYLOBACTER

Name of farm _____ **Broiler house** _____

ID

- 1** Write the identifying information in full (including the date of sampling and the age of the birds) on the label of the empty plastic bag. Please ensure that the correct sample kit is used for the age of birds sampled.
- 2** Individual cloacal swabs should be taken from 16 birds in the study broiler house. Four birds should be chosen from different areas in each quarter of the house so that a total of 16 birds are swabbed.
- 3** If possible, get someone to hold the birds for you to swab.
- 4** To obtain a cloacal swab, remove a sterile swab from its wrapping and insert the tip gently into the cloaca. Rotate in both directions and gently remove. Remove the cap from the tube of medium and place the swab into the medium pushing the swab cap firmly into the tube.
- 5** Put all 16 cloacal swabs in the labelled plastic bag and seal the bag. Place left over wrapping and caps in the other plastic bag and suitably dispose.
- 6** Place the sealed plastic bag of cloacal swabs in the return jiffy bag and seal securely.
- 7** Write the farm address on the back of the jiffy bag and post the same day to CVL.
- 8** The same broiler house should be sampled on each occasion.

In case of queries, please contact Mrs Sarah Evans MRCVS, Epidemiology Department, Central Veterinary Laboratory, New Haw, Addlestone, Surrey KT15 3NB

Tel: 0932 341111 ext. 2459

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APPENDIX C.4

Instructions for slaughter sampling (surveyed flock)

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CVL CAMPYLOBACTER BROILER STUDY

INSTRUCTIONS FOR SLAUGHTER SAMPLING AT FINAL DEPOPULATION OF BROILER HOUSE

Name of farm _____ Broiler house _____

ID SL

- 1 Write the identifying information in full (including the date of sampling and the age of the birds) on the label of the empty plastic bag.
- 2 Please sample 16 birds from the last batch of birds to be slaughtered from the study broiler house (final depopulation).
- 3 Cloacal swabs should be taken from the birds immediately prior to slaughter at the processing plant eg. at shackling or, if more convenient, sampling can be conducted at the farm on the day or day before slaughter.
- 4 To obtain a cloacal swab, remove a sterile swab from its wrapping and insert the tip gently into the cloaca. Rotate in both directions and gently remove. Remove the cap from the tube of medium and place the swab into the medium pushing the swab cap firmly into the tube.
- 5 Great care should be taken to obtain cloacal swabs without the swab touching the feathering of the bird or any equipment in order to avoid cross-contamination when samples are taken at the processing plant.
- 6 Put all 16 cloacal swabs in the labelled plastic bag and seal the bag. Place left over wrapping and caps in the other plastic bag and suitably dispose.
- 7 Place the sealed plastic bag of cloacal swabs in the return jiffy bag and seal securely.
- 8 Write the farm address on the back of the jiffy bag and post the same day to CVL.

In case of queries, please contact Mrs Sarah Evans MRCVS, Epidemiology Department,
Central Veterinary Laboratory, New Haw, Addlestone, Surrey KT15 3NB

Tel: 0932 341111 ext. 2459

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APPENDIX C.5

Cleansing and disinfection questionnaire

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CAMPYLOBACTER BROILER STUDY

CLEANSING AND DISINFECTION QUESTIONNAIRE

Mrs S J Evans MRCVS,
Epidemiology Department, Central Veterinary Laboratory,
New Haw, ADDLESTONE, Surrey KT15 3NB
Tel: 0932 341111 Ext. 2459

TO BE COMPLETED AND RETURNED AS SOON AS POSSIBLE

Name of farm _____ Study broiler house _____

Your name _____ Today's date _____

Please give a full account of the method of cleaning and disinfection used to clean out the study broiler house after the last production cycle on the form below. Please send a copy of the clean out protocol used, if available.

Please complete and return this questionnaire to CVL as soon as possible after clean out.

For the study broiler house, give the dates:-

the last batch of birds were slaughtered: _____

the new crop of chicks were/will be placed: _____

the expected date of next final depopulation of the house: _____

C	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ID	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	/ /		
	/ /		
	/ /		
1	<input type="checkbox"/>		
2	<input type="checkbox"/>		
3	<input type="checkbox"/>	<input type="checkbox"/>	
4	<input type="checkbox"/>		
5	<input type="checkbox"/>		
6	<input type="checkbox"/>		
7	<input type="checkbox"/>		
8	<input type="checkbox"/>		
9	<input type="checkbox"/>		

SECTION 1 : Broiler House Construction

1. Type of building: Controlled ventilation broiler shed

 Other (specify) _____

2. Age of building (years) _____

3. Size of house: Length: _____ feet Width: _____ feet

4. Floor construction: Concrete Rammed chalk Earth

 Other (specify) _____

5. Wall construction: Concrete block (full height) Brick

 Boarding on concrete block Timber

 Other (specify) _____

6. Ventilation System: Natural Roof fans Side fans Ducted

7. Source of birds water: Mains Borehole Well River

8. Water drinkers: Cup Nipple Bell

 Other (specify) _____

9. Feeding system: Hopper Pan Chain

 Other (specify) _____

SECTION 1 : (continued)

10. Does the house have waterproof electrics? No Yes
11. What repairs are required to
 a) the house: _____
 b) the equipment: _____
12. How much of the house is surrounded by a concrete apron (eg none, ends only, all)?

10

11

12

SECTION 2: House Cleaning

13. Who cleaned the broiler house? Staff Company team Contractor
14. What was the method of dust removal from beams, ceiling etc.?
 Swept Blown Vacuum Wet wash
 Other (specify) _____
15. Which of the following areas were dusted?
 Ledges Fan shafts Vents Ceiling
16. How was the dirt, including the litter removed? _____
17. How was the litter disposed? _____
18. Was disinfectant applied before the litter was removed? No Yes
19. What was the method of cleaning?
 Spray Power wash Steam clean
 Other (specify) _____
20. Were detergents/sanitizers used during cleaning? No Yes
 If YES: Name of manufacturer _____
 Name of product _____
 Concentration used _____
 Amount used _____
21. Which of the following areas were wet-cleaned?
 Fan shafts Vents Full height walls Ceiling

13

14

15

16

17

18

19

20

21

SECTION 2 : (continued)

22. How was the wash water disposed? _____

22

23. Give a score from 1-5 to indicate the effectiveness of the cleaning, where 1 is the worst score (copious dirt remaining on surfaces) and 5 is the highest score (extremely clean surfaces throughout house).

23

AREA	FLOOR 1 - 5	BEAMS 1 - 5	FANS 1 - 5	FEED HOPPER 1 - 5	ANTE-ROOM 1 - 5
Give ONE score for each area	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SECTION 3: House Disinfection

24. Who disinfected the broiler house? Farm staff Company team Contractor

24

25. When was the house disinfected?

During wet-cleaning After washing: House still wet House dry

25

If AFTER WASHING: What was the time period between washing and disinfection?

26. How was disinfectant applied? _____

26

27. Give the name of the disinfectant/s used

27

	Product 1	Product 2
Name of manufacturer	_____	_____
Name of product	_____	_____
Concentration used	_____	_____
Amount used	_____	_____

27.1	27.2
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

28. Was the house fogged/fumigated after disinfection? No Yes

28

If YES: Name of manufacturer _____
 Name of product _____
 Concentration used _____
 Amount used _____
 How applied _____
 Number of times applied _____

SECTION 3 : (continued)

29. What was the time period between completion of disinfection and:

a) replacement of litter? _____ b) repopulation? _____

29

SECTION 4: Equipment and Other Cleansing and Disinfection

30. What was the method of cleansing and disinfection, including whether dismantled, of

Header tank: _____

Water lines: _____

Feed hoppers: _____

Feed lines: _____

30

31. Where was equipment cleaned? _____

31

32. Where was equipment stored? _____

32

33. When was equipment replaced in the house? _____

33

34. Is a water sanitizer added to drinking water? No Yes

34

If YES: Name of manufacturer _____

Name of product _____

Concentration used _____

When used _____

35. During the clean out procedure, is the:- No Yes

Stores area: cleaned?

disinfected?

Area outside shed: cleaned?

disinfected?

35

36. Following which procedure is a boot dip used at the shed entry?

Disinfection of house Litter arrival Chick arrival At all times

36

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE. PLEASE RETURN TO THE CENTRAL VETERINARY LABORATORY IN THE ATTACHED REPLY-PAID ENVELOPE.

APPENDIX C.6

Rodent questionnaire

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Original

CAMPYLOBACTER BROILER STUDY

RODENT QUESTIONNAIRE

Mrs S J Evans MRCVS,
Epidemiology Department, Central Veterinary Laboratory,
New Haw, ADDLESTONE, Surrey KT15 3NB
Tel: 0932 341111 Ext. 2459

C	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ID	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

TO BE COMPLETED WHEN THE BIRDS ARE 5-6 WEEKS OF AGE AND RETURNED WITH THE DISEASE SECURITY QUESTIONNAIRE AT THE END OF THE PRODUCTION CYCLE.

Name of farm _____ Study broiler house _____
Your name _____ Today's date _____

Please follow the instructions given to carry out a thorough survey of the study broiler house and surrounding area for the presence of rodents and give full details of methods of rodent control employed by the site. Please send copies of recent pest contractors reports for the site, if available.

SECTION 1 : Rodent Survey

Use the following description of the traces and signs of rodent activity to assess the level of infestation of rodents in and around the study broiler house when the birds are between 5 and 6 weeks of age.

Traces and Signs of Rodent Activity

Droppings

Mice will leave copious quantities of droppings throughout their territories and these will be found on all horizontal surfaces, amongst materials and on ledges or other movement routes.

Holes and runs

Holes are chewed in cladding or other vulnerable materials particularly, at intersections of walls and ceilings or behind structures such as control panels and feeders. Burrowing in litter or droppings accumulations can be identified by the presence of small tunnel entrances and foot prints may be observed on runways or other movement areas, where dust accumulates. Rat burrows are typically 3-4" in diameter.

Smear marks and urine pillars

The natural grease on the bodies of rodents will attract dirt and in turn, this will be deposited on pathways or other structures such as beams which rats and mice are using regularly. These appear as dark smears. Mice will urinate at specific locations and when combined with dust or other solid materials this will lead to the creation of small pillars which are often found on pipes, cables and ledges which are used as main movement routes. Heavy mouse infestations produce a pungent odour due to urine deposition in their territories and this can identify the general location of populations.

Damage

Gnawing can cause damage to building structures particularly cladding and insulation and associated equipment and chewed materials can be found when accumulations of rubbish or other vulnerable items such as bales of wood shavings or paper sacks are investigated.

1. Please give a score from 1 to 5 for each sign of rodent activity, where 1 is the lowest score (no evidence) and 5 is the highest score (large numbers or amount present).

	BROILER HOUSE	FEED HOPPER/STORE AREA
1.1 MICE SIGNS	1 - 5	1 - 5
Droppings	<input type="checkbox"/>	<input type="checkbox"/>
Holes/runs	<input type="checkbox"/>	<input type="checkbox"/>
Smear marks	<input type="checkbox"/>	<input type="checkbox"/>
Urine pillars	<input type="checkbox"/>	<input type="checkbox"/>
Damage	<input type="checkbox"/>	<input type="checkbox"/>

(Give ONE score for each sign)

(Give ONE score for each sign)

1.1

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

SECTION 1 : (Continued)

BROILER HOUSE

FEED HOPPER/STORE AREA

1.2 RAT SIGNS

1 - 5

1 - 5

Droppings

Holes/runs

Damage

(Give ONE score for each sign)

(Give ONE score for each sign)

2. Estimate the number of live and dead rodents seen in and around the study broiler house during the last 7 days.

NUMBER SEEN

NUMBER SEEN

MICE Live : _____

Dead : _____

RATS Live : _____

Dead : _____

1.2

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

2

2.1

2.2

SECTION 2: Rodent Control

3. Who currently undertakes rodent control on the site?

Own staff

Contractor

If CONTRACTOR, give name of company used _____

4. When are rodent control measures taken?

All year round

At depopulation

When infestations build up

5. What types of rodent control are currently used?

Bait points

Traps

Cats

Other (specify) _____

6. Methods of rodent control (if applicable)

BAIT POINTS

TRAPS

No. of bait points/traps in house _____

How often serviced/replenished _____

Bait used: Company name _____

Product name _____

No. of rodents killed per week _____

Type of trap used _____

6

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE. PLEASE RETURN TO THE CENTRAL VETERINARY LABORATORY IN THE ATTACHED REPLY-PAID ENVELOPE.

APPENDIX C.7

Disease security questionnaire

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In
Original

DISEASE SECURITY QUESTIONNAIRE

Mrs S J Evans MRCVS,
Epidemiology Department, Central Veterinary Laboratory,
New Haw, ADDLESTONE, Surrey KT15 3NB
Tel: 01932 341111 Ext. 2459

TO BE COMPLETED AND RETURNED AT THE END OF THE PRODUCTION CYCLE

Tick all boxes that apply and give written details using CAPITAL LETTERS

SECTION 1 : Identifying Information

Name and address of farm _____

Post Code _____

Telephone No. (incl. code)

Type of farm : Broiler Company managed Contract (privately owned)

Name and address of Processing Plant _____

Distance from farm to Processing Plant _____ miles

Details of person completing the questionnaire:

Your name _____ Today's date _____

Your position : Owner Manager Farms Manager (Broiler Company)

Other (specify) _____

SECTION 2 : Summary of Site and Management (tick ALL that apply)

1. Apart from the broiler flock, are there any other poultry on the farm?

No Yes: Fowl: Layers Brooders

Other species: Turkeys Ducks

2. Are there any other species of domestic animals on the farm?

No Yes: Cattle Sheep Pig

Horse Dog Cat

Other (specify) _____

3. Do cattle, sheep or pigs have access to the area directly surrounding the broiler houses, eg. for grazing

No Yes

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C

D

1

2

3

/ /

4

5

6

SECTION 3 : Disease Security at the Site

NO YES

1. Is the site secured by a continuous perimeter fence?
2. Is there MORE THAN ONE entrance to the site?
3. Is there a warning notice at the site entrance?
4. Do vehicles drive through a disinfectant wheel bath on entry?

5. How far is it to the nearest neighbouring farm with poultry? _____ miles

6. Please give the number of personnel working at the site:
 a) Full time _____ b) Part time _____

YES:
 CHICK DELIVERY ONLY AT OTHER TIMES
 NO

7. Since the current crop of chicks were placed (delivered):
 a) have any personnel helped at other poultry sites?
- b) have any workers from other poultry sites helped at this site?

8. What hygiene facilities are used by personnel?

None Wash basin Shower

9. Do any personnel keep poultry, pigeons or pet birds at home? NO YES

10. What protective clothing is worn by personnel?

Waterproof footwear Overalls Gloves Hat Mask

11. Is there a disinfectant boot dip outside the house?

No Yes : In food store Yes : Outside building

12. Give the name of the disinfectant in the boot dip and concentration used : Company name _____

Product name _____

Concentration used _____

13. How frequently is the disinfectant solution in the boot dip changed?

14. Is an alcohol rinse or bactericidal soap used by personnel when hand washing?

No Yes

15. Dead bird disposal : On site : Burial Composted Incinerated

Off site : Removed by a Contractor Knackers

8
 9
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 22
 23
 24

SECTION 4 : House by House Information (for the current production cycle)

Give details of ALL broiler houses on the site

* A house is a building or part of a building with solid partitions and its own ventilation system.
 † A shared building is a building which contains more than one individual "house".

	1	2	3	4
House* name or number				
Shared building † (Yes/No)				
Number of chicks placed				
Date(s) of chick delivery				
Date(s) of slaughter (give all dates if house depopulated in batches)	Date 1			
	Date 2			
	Date 3			
	Date 4			

	5	6	7	8
House* name or number				
Shared building † (Yes/No)				
Number of chicks placed				
Date(s) of chick delivery				
Date(s) of slaughter (give all dates if house depopulated in batches)	Date 1			
	Date 2			
	Date 3			
	Date 4			

	9	10	11	12
House* name or number				
Shared building † (Yes/No)				
Number of chicks placed				
Date(s) of chick delivery				
Date(s) of slaughter (give all dates if house depopulated in batches)	Date 1			
	Date 2			
	Date 3			
	Date 4			

IN CONFIDENCE

CAMPYLOBACTER BROILER STUDY

SECTION 4 (Contd)

Give details of ALL broiler houses on the site

- * A house is a building or part of a building with solid partitions and its own ventilation system.
† A shared building is a building which contains more than one individual "house".

		13	14	15	16
House* name or number					
Shared building † (Yes/No)					
Number of chicks placed					
Date(s) of chick delivery					
Date(s) of slaughter (give all dates if house depopulated in batches)	Date 1				
	Date 2				
	Date 3				
	Date 4				

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SECTION 5 : Withdrawal Ratios

For the study broiler house ONLY, give the LAST DATES on which the chicks were receiving rations containing

1. Antibiotic _____
2. Growth Promotant _____
3. Coccidiostat _____

Study broiler house name or number _____

/ /

/ /

/ /



THANK YOU FOR COMPLETING THIS QUESTIONNAIRE. PLEASE RETURN TO THE CENTRAL VETERINARY LABORATORY IN THE ATTACHED REPLY-PAID ENVELOPE.

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