

SALMONELLA IN LIVESTOCK PRODUCTION IN GB 2008

INTRODUCTION

This publication presents data on *Salmonella* reports from livestock species in Great Britain (England, Wales and Scotland) collected and collated by the Department for Environment, Food and Rural Affairs (Defra) during 2008 and also provides data from previous years for comparative purposes. The data in the first five chapters cover the reason for sampling and place of sampling, reports of *Salmonella* in livestock with separate sections for the main species covered, reports of *Salmonella* in wildlife, reports of *Salmonella* in livestock products and reports of *Salmonella* in animal feedingstuffs. The sixth chapter covers the antimicrobial sensitivity of *Salmonella* (England and Wales only).

Since 1993 the date of a *Salmonella* incident has been recorded as the date it was reported to an Officer of the Minister. Under the present system any *Salmonella* reports that are confirmed or identified after the publication of the annual report will be incorporated into the revised tables that appear in the following year's publication. This may cause the number of incidents and/or isolations to differ from that previously given for a particular year. The most recent version of the report should therefore always be used when comparing incidents from year to year.

Revisions in the way that data have been compiled and presented since 1993 mean that, with the exception of the tables on *Salmonella* in animal feedingstuffs, data in this report cannot be compared directly with information published prior to 1993. A more detailed comparison can be generated, if required, for any *Salmonella* serovar, phage type (in the case of *Salmonella* Enteritidis, *S. Hadar*, *S. Pullorum*, *S. Thompson* and *S. Virchow*) or definitive phage type (in the case of *S. Typhimurium*). Requests for such data should be made to the Centre for Epidemiology and Risk Analysis (CERA), Veterinary Laboratories Agency, Weybridge who will be happy to assist with requests (s.a.kidd@vla.defra.gsi.gov.uk).

Care should be taken when comparing data from one year to another as an increase or decrease in the number of incidents and isolations does not necessarily indicate a similar change in prevalence. This is because the total number of samples examined and their distribution are not known.

A new feature this year is the introduction of maps illustrating the number of incidents of *Salmonella* and the density of registered premises in Great Britain by species. Maps are provided for cattle, sheep, pigs, chickens, turkeys and ducks and may be found after the relevant species tables.

STATUTORY ASPECTS OF *SALMONELLA* CONTROL IN GREAT BRITAIN

On 1st March 1989 the Zoonoses Order 1975 was revoked and replaced by the Zoonoses Order 1989. The 1989 Order added horses, deer and pigeons to the range of species from which *Salmonella* isolations are subject to reporting¹. Under the 1989 Order the responsibility for reporting the isolation of a *Salmonella* was placed on the laboratory carrying out the examination or in the case of examinations elsewhere, the person carrying out the examination. In practice, reports of *Salmonella* isolations must be made to the Nominated Officer at one of the Regional Laboratories of the Veterinary Laboratories Agency (VLA) or to a Divisional Veterinary Manager in Scotland. If so required, the Department must be provided with a culture of the organism.

The requirement to test poultry for *Salmonella* on a regular basis under the Poultry Laying Flocks (Testing and Registration etc.) Order 1989 and the Poultry Breeding Flocks & Hatcheries (Registration and Testing) Order 1989, increased the number of examinations carried out from 1989 onwards. These two Orders were revoked in 1993 with the implementation of the Poultry Breeding Flocks and Hatcheries Order (PBFHO) 1993, which brought *Salmonella* control measures in poultry into line with the European Union Directive 92/117/EEC, with the result that the level of monitoring in some poultry sectors altered. This needs to be borne in mind when examining long-term data for poultry. This Order only applied to chickens in its requirement for regular monitoring of breeding flocks and hatcheries using methods laid down in the Order. In 2007 the PBFHO 2007 replaced the PBFHO 1993 and set out the requirements for registration and sampling in a new *Salmonella* National Control Programme (NCP) for chicken breeding flocks. According to the new Order, statutory testing of breeding flocks of domestic fowl

¹ *Salmonella* isolations from the following species must be reported to an Officer of the Minister: cattle, sheep, goats, pigs, rabbits, horses, deer, domestic fowl (chickens), turkeys, ducks, geese, guinea fowl, pheasants, partridges, pigeons and quail.

during the rearing phase and during the period of production of eggs for hatching takes place on the breeding flocks holding only, and an enhanced sampling (boot swabs or composite faeces) and detection method using Modified Semi-Solid Rappaport Vassiliadis culture medium (ISO 6579: Annex D) is used. The modified sampling protocol specified by the PBFHO 2007 is not directly comparable with PBFHO 1993. The PBFHO 2007 was in turn revoked and replaced by the Control of Salmonella in Poultry Order (CSPO) 2007, which came into force on 28th January 2008 and included the requirements for the implementation of a NCP in commercial laying flocks and commercial breeding flocks. Please refer to Chapter 2.5 (Salmonella in Poultry) for further information.

The European Council Directive 2003/99/EC provides the statutory basis for monitoring of zoonoses and zoonotic agents in the EU. Member States are required to monitor certain zoonoses and to report to the Commission each year the trends and sources of those zoonotic infections. This covers animals, feed, food and the relevance to human infection, as well as trends in antimicrobial resistance in *Salmonella*, *Campylobacter* and other indicator organisms. The Zoonoses Regulation 2160/2003 came into force on 21st December 2003. The aim of the Regulation is to reduce the prevalence of certain zoonotic infections at the primary production level, by establishing the level in the Community and setting a target for the reduction of the level. As a result each Member State is required to produce a plan to achieve the target. Further details on the mandatory surveys in flocks of laying hens, broiler flocks and turkey flocks are included in the Poultry Chapter.

DEFINITION OF AN INCIDENT AND ISOLATION

In contrast to *Salmonella* in humans, many isolations of *Salmonella* from livestock are not associated with clinical disease, or occur on farm premises in which *Salmonella* has been isolated from a group of animals rather than an individual. Since 1993 reports of *Salmonella* from livestock have been separated into isolations and incidents. "Isolations" comprise individual reports of *Salmonella* made from samples and reported to Officers of the Minister. "Incidents" afford a truer picture of the amount of *Salmonella* in the animal population as they do not include repeat isolations of a serovar that may result from a number of samplings during the course of an investigation, or monitoring activities on a particular premises. Isolates, isolations and incidents are defined in the following way:

An isolate is a single culture of a particular *Salmonella*, and results from a single sample.

An isolation is defined as the report of the first isolate of a given *Salmonella* (defined by serovar, and/or phage type, if available) from the same group of animals on a given occasion. If two submissions from the same group of animals on different dates give the same serovar, this is reported as two isolations.

An incident comprises the first isolation and all subsequent isolations of the same serovar or serovar and phage/definitive type combination of a particular *Salmonella* from an animal, group of animals or their environment on a single premises, within a defined time period (usually 30 days).

The first such report of any particular serovar or serovar and phage/definitive type combination of *Salmonella* from a particular animal, group of animals or their environment will therefore be recorded as one incident and one isolation. Further reports of the same *Salmonella* from the same group during the incident investigation will be recorded as further isolations, but not as further incidents unless the isolation is from an epidemiologically distinct group of animals. Examples of this would include a distinct group of the same species on a separate part of the same premises. Reports of a different serovar or phage/definitive type of *Salmonella* from the same animals will be recorded as a new incident. Thus two reports of *S. Typhimurium*, one of DT104 and another of DT193, from the same group of animals would count as one incident and one isolation of *S. Typhimurium* DT104 and one incident and one isolation of *S. Typhimurium* DT193, whilst two reports of *S. Typhimurium* DT12 from the same group of animals would count as one incident but two isolations.

Reporting on the progress of the *Salmonella* NCPs is done using different methods and does not involve the reporting of incidents, but rather reporting of “positive flocks”.

In 2006 improvements were made to the functioning of the program that allocates incidents within the *Salmonella* Database and these have resulted in changes in the number of incidents and isolations recorded, particularly with reports of those serovars that are phage typed (*S. Typhimurium*, *S. Enteritidis*, *S. Hadar*, *S. Pullorum*, *S. Thomson* and *S. Virchow*). The effect of this is that some isolations which were previously linked together to form an incident are no longer linked and are reported as isolations only.

Starting with samples collected from 1st January 2006, any hatchery isolates where there are no supply flock details available are now treated as isolations only and not incidents as they cannot be traced back to a specific flock. The species that are mainly affected by this improvement are chickens and ducks.

From the start of implementation of the NCP in 2008, data on positive findings of *Salmonella* in laying and breeding chicken flocks, is reported as number of positive flocks, as required by the legislation, as well as number of positive incidents detected during the year. The number of reported incidents of *Salmonella* detection in chickens does not equate directly to the overall number of positive flocks that are detected during the year. A flock is counted as positive only once, irrespective of the number of incidents occurring and the multiplicity of serovars determined.

The concept of an "incident" is clearly inappropriate when referring to isolations from animal feedingstuffs or human foodstuffs of animal origin, so data for these are only reported in terms of isolations of *Salmonella*. With this in mind the tables reporting the reason for sampling and the place from which the sample originated have been divided to indicate the number of incidents for samples taken at premises other than slaughterhouses, and the number of isolations for samples taken at slaughterhouses or human food premises.

All samples that have been taken in England and Wales are sent to a VLA Laboratory for examination and confirmation of *Salmonella*. Of those samples taken in Scotland, the majority of poultry samples are sent to VLA Lasswade and all mammalian samples are sent to the Scottish Agricultural College (SAC) and confirmed by Health Protection Scotland (HPS).

Data from research projects and surveys are reported separately and are excluded from the tables in the species chapters in this publication, however details of any serovars reported from animals through research projects or surveys and not reported through routine (scanning) surveillance are highlighted in the relevant species chapters. The antimicrobial sensitivity chapter (Chapter 6) contains data from routine surveillance and other surveillance projects.

SEROTYPING AND PHAGE TYPING METHODS

Salmonella isolated from animals and feed is biochemically confirmed and serotyped by micro, tube and slide agglutination tests. Each culture is tested for the presence of somatic and flagella antigens by mixing with specific *Salmonella* antisera. Where homologous antiserum and antigen react, clumps of bacteria form as visible agglutination. Serovars are derived by reference to the Kauffmann White Scheme. Additional biochemical tests are needed to confirm some serovars. *Salmonella* Typhimurium, *S. Enteritidis*, *S. Hadar*, *S. Thompson*, *S. Virchow* and

S. Pullorum are phage typed according to the Health Protection Agency phage typing schemes. Cultures are seeded onto special agar plates and a specific set of phages applied to the culture. After incubation the degree and pattern of lysis is read and a phage type or definitive type (for *S. Typhimurium*) attributed to the culture (Anderson and others 1977, Ward and others 1987).

Serotyping and phage typing of samples received from premises in England and Wales are done by the VLA. Mammalian, and some poultry samples, from Scotland are serotyped and phage typed by HPS and the majority of poultry samples from Scotland are serotyped and phage typed by VLA.

Some phage types are 'related variants' although they are still reported as distinct types, eg. PTs 4 and 7 of *S. Enteritidis* and DT12, DT104, DT104b and U302 of *S. Typhimurium*.

METHODS USED FOR SCREENING *SALMONELLA* VACCINE STRAINS

Following the introduction of live vaccines for *Salmonella* Enteritidis and *Salmonella* Typhimurium, additional testing is required to distinguish field strains from vaccine strains.

S. Enteritidis and relevant *S. Typhimurium* isolates are compared to the *Avipro Vac E* and *Vac T* vaccine strains, which carry antibiotic resistance markers, using a panel of four relevant antibiotics in a disc diffusion technique. If the test strain result is similar to a vaccine strain, confirmatory tests using agar plates containing relevant antibiotics are carried out.

Both *Avipro Vac E* and *Avipro Vac T* are sensitive to Erythromycin and resistant to Rifampicin to distinguish them from *Salmonella* field strains. To differentiate between the two *Avipro* vaccine strains, *Vac E* has an additional resistance to Streptomycin and *Vac T* has an additional resistance to Nalidixic acid.

Gallivac SE vaccine has no resistance markers but contains mutations causing auxotrophism for histidine and adenine. *S. Enteritidis* isolates are compared to the vaccine strain by growth on minimal media with and without histidine and adenine.

The disc diffusion test using the supplementary panel of four antibiotics is carried out at VLA Weybridge and Lasswade. Confirmatory tests for *Vac E* and *Vac T* vaccine strains and *Gallivac SE* vaccine tests are carried out at VLA Weybridge.

NOMENCLATURE

The nomenclature used throughout this publication follows that devised by Le Minor and Popoff which divides the bacterial genus *Salmonella* into two species, *Salmonella enterica* and *Salmonella bongori*. The species *Salmonella enterica* is divided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*.

The method of naming serovars of subspecies *enterica* differs from that used for the other five subspecies in that the familiar serovar names are assigned to subspecies *enterica* whilst the other subspecies are designated by antigenic structure.

For example, following this method the serovar originally referred to as *Salmonella typhimurium* is now known as *Salmonella enterica* subspecies *enterica* serovar Typhimurium which may be shortened to *Salmonella* Typhimurium and the naming of serovars of subspecies *diarizonae* is, for example, *Salmonella enterica* subspecies *diarizonae* serovar 61:k:1,5,7 (or *Salmonella* III 61:k:1,5,7). For further details of this nomenclature see: Le Minor, L, Popoff, MY (1987).

The serovar formally known as *Salmonella* Java has now been reclassified, on the basis of genetic similarity studies, as *Salmonella* Paratyphi B variant (var.) Java. It is a group B *Salmonella* and has the same antigenic structure as *Salmonella* Paratyphi B (O = 4, 12: H = b: 1.2). *Salmonella* paratyphi B var. Java and *Salmonella* Paratyphi B are differentiated by the dextro-tartrate test, in which *Salmonella* Paratyphi B var. Java gives a positive acid reaction, whereas *Salmonella* Paratyphi B is negative.

SALMONELLA DATABASE

In January 2003 the new VLA integrated *Salmonella* recording and reporting system went live. *Salmonella* data and test results are entered into the VLA networked FarmFile database, which contains baseline data for all VLA submissions. Data entry has been decentralised in England and Wales and details for all submissions, except those received as *Salmonella* isolates from avian species from private laboratories for typing (direct avian submissions), are now entered at the Regional Laboratory receiving the submission. The VLA Sample Manager and Laboratory Information Management IT systems have been extended to incorporate the results of tests carried out on *Salmonella* isolates (group, serovar, phage type and antimicrobial resistance testing). The system has replaced both the *Salmonella* incident recording system (ZO2 database) and the antimicrobial sensitivity database (Sentest). Automatic e-mail alerts are sent to

relevant personnel when a *Salmonella* of potential public health importance is isolated, and the VLA Regional Laboratories and Government Veterinary Advisers have the ability to view data directly.

The database was developed with the aim of facilitating the linkage of related data, to reduce the paper flow involved in reporting isolations and to avoid duplication of data recording and consequently reduce the potential for errors. The overall objectives were to improve the quality and timeliness of the information obtained from *Salmonella* cases, to make the data more accessible to those involved with deciding and implementing control policies, and to allow enhanced epidemiological analysis of the data.

Under the previous data recording systems an isolation was flagged as a new incident by the Nominated Officer dealing with the report, however the new *Salmonella* database automatically designates new incidents using a series of pre-defined criteria which are matched against those reported for previous isolations in the database where a sample or culture is submitted to VLA. The criteria interrogated are: date (within 30 days of the previous isolation), serovar (and phage type if available), animal species, flock type (poultry) and the location from which the sample was taken. If the Nominated Officer indicates that the automatically-designated incident reference is not correct, for example, if he/she is aware that an isolation made more than 30 days after a previous isolation of the same serovar is, in fact, part of an ongoing incident, or that two isolations were made from different epidemiological groups, a 'manual over-ride' function can be used to ensure that the incident is identified correctly on the database. Previous reports therefore include incidents as defined by the Nominated Officer, for submissions received until 31st December 2002 and incidents generated automatically, for submissions received from 1st January 2003 onwards.

EARLY DETECTION SYSTEM

A system for the early detection of outbreaks of *Salmonella* in livestock in Great Britain was implemented in 2006. The system consists of a regression model developed for incidents of *Salmonella* from clinically diseased domestic livestock species and for reports received from poultry monitored under the Poultry Breeding Flocks and Hatcheries Order 2007 and the Control of Salmonella in Poultry Order 2007. An expected and threshold value is derived on a monthly basis by fitting the regression model, which accounts for seasonality and past outbreaks, in the dataset. If the current observed count is above the estimated threshold value, a warning is implemented indicating that a potential outbreak is occurring in the field. This initiates appropriate investigations. This model is complemented by a monthly tool which

aims at the detection of certain new or unusual *Salmonella* serovars reported from animals.