INTRODUCTION
This publication presents data on *Salmonella* reports from livestock species in Great Britain (England, Wales and Scotland) collected and collated by the Animal and Plant Health Agency (APHA) during 2016 and also provides data from previous years for comparative purposes. The data in the first eleven chapters cover reports of *Salmonella* in livestock, with separate chapters for the main species, reports of *Salmonella* in wildlife and reports of *Salmonella* in animal feedingstuffs. The twelfth chapter covers the antimicrobial susceptibility 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 result in the number of incidents and/or isolations differing from that previously given for a particular year. The most recent version of the report should therefore always be used when comparing data 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, or phage type in the case of *S. Enteritidis* and *S. Typhimurium*. Requests for such data should be made to the Department of Epidemiological Sciences, APHA Weybridge who will be happy to assist with requests (sue.kidd@apha.gsi.gov.uk).

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

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On 1st March 1989 the Zoonoses Order 1975 was revoked and replaced by the Zoonoses Order 1989.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 reporting. 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 a Veterinary Investigation Officer at one of the Veterinary Investigation Centres (previously Regional Laboratories) of the APHA or to a Regional Veterinary Lead in Scotland. A culture of the organism must be made available on request.

From the late 1980s, there have been statutory *Salmonella* control programmes for certain sectors of the poultry industry in the UK. These controls have been amended over the years. 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. Zoonoses Directive (EC) No. 92/117 required Member States to monitor the trends and sources of various zoonotic agents in animals, feed, food and people, analyse them and report the findings to the Commission. In addition, it required Member States to monitor breeding flocks of domestic fowl (*Gallus gallus*) for *Salmonella*. If *S. Enteritidis* or *S. Typhimurium* was confirmed to be present in a breeding flock then the flock was slaughtered. The monitoring of breeding flocks took place at hatcheries with follow up confirmation in the birds on the farm.

A review of Directive (EC) No. 92/117 was carried out in the late 1990s by the Scientific Committee on Veterinary Measures relating to Public Health, and in its Opinion published in April 2000 it was considered that the measures in place at that time to control food-borne zoonotic infections were insufficient. The Committee went on to propose other risk management options. As a result, in 2003, Member States agreed that the monitoring of specified zoonotic agents should be expanded and harmonised, where beneficial, in a new Directive (EC) No. 2003/99 and that the risk management measures required to control zoonotic infections should be extended in a new Regulation (EC) No. 2160/2003.

The European Council Directive 2003/99/EC currently 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 Directive covers animals, feed, food and the
relevance to human infection, as well as trends in antimicrobial resistance in \textit{Salmonella}, \textit{Campylobacter} and other indicator organisms.

The Zoonoses Regulation (EC) No. 2160/2003 came into force on 21\textsuperscript{st} 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 reduction. As a result, each Member State is required to produce a programme to achieve the target.

In order to implement Regulation (EC) No. 2160/2003, the PBFHO 2007 replaced the PBFHO 1993 and set out the requirements for registration and sampling for a new \textit{Salmonella} National Control Programme (NCP) for chicken breeding flocks. According to the new Order, statutory testing of breeding flocks of domestic fowl during the rearing phase and during the period of production of eggs for hatching takes place on the breeding flock 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; now incorporated into ISO6579-1/17) 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 \textit{Salmonella} in Poultry Order (CSPO) 2007 which came into force in January 2008 and included the requirements for the implementation of a NCP in commercial laying flocks, together with that already in place for breeding chicken flocks. In 2009, the Control of \textit{Salmonella} in Broiler Flocks Order 2009 came into force in England and Wales, and in Scotland the CSPO 2007 was revoked and replaced by the Control of \textit{Salmonella} in Poultry (Breeding, Laying and Broiler Flocks) (Scotland) Order 2009. This legislation implemented the requirement for a \textit{Salmonella} National Control Programme in the broiler chicken sector.

In January 2010, the Control of \textit{Salmonella} in Turkey Flocks Orders 2009 came into force in England and Scotland, and in February 2010 the Control of \textit{Salmonella} in Turkey Flocks Order 2010 came into force in Wales. This legislation enforces Regulation (EC) No. 2160/2003 and Regulation (EC) No. 1190/2012 and implements the requirement for a \textit{Salmonella} National Control Programme in the turkey sector. The Order makes provision for the testing of turkey flocks for \textit{Salmonella}. As with the NCP in chicken flocks, it also prohibits the use of antimicrobials to control non-clinical Salmonella and live \textit{Salmonella} vaccines that cannot be distinguished from field strains.
The above changes in legislation and subsequent levels of monitoring for *Salmonella* in the GB commercial chicken and turkey sectors need to be borne in mind when examining long-term data for poultry. It should also be noted that the poultry industry is currently the only food animal production sector that has structured bacteriological surveillance programmes for *Salmonella* in place. This routine monitoring may be expected to result in larger numbers of *Salmonella* isolates than the scanning surveillance of diagnostic submissions that applies to other farm livestock. Please refer to Chapter 6 (Chickens) and Chapter 7 (Turkeys) for further information.

**DEFINITION OF AN ISOLATION AND INCIDENT**

In 2012, changes were made to the tables and figures of this publication such that, for all species not covered by a National Control Programme (NCP), precedence is now given to the number of isolations rather than the number of incidents. This is because the number of isolations gives a more representative picture of the number of *Salmonella* isolates reported in livestock, however incidents are still useful for epidemiological purposes.

Chapters 6 and 7 (chickens and turkeys, respectively, both of which are covered by NCPs) focus on the number of flocks from which the various *Salmonella* serovars have been reported and show these data together with the number of isolations. Incidents are no longer reported in this publication for chickens and turkeys.

Since the implementation of the NCPs for chicken breeding, laying and broiler flocks in 2007, 2008 and 2009, respectively, the data on positive findings of *Salmonella* in laying, breeding and broiler chicken flocks have been reported as the number of positive flocks, as required by the legislation, as well as the number of positive isolations detected during the year. This is also the case in turkey flocks, for which the NCP was implemented in 2010. The number of reported isolations of *Salmonella* detected in chickens and turkeys 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 isolations occurring and the number of serovars identified.

As the tables and figures of Chapter 1 present combined data for cattle, chickens, ducks, pigs, sheep and turkeys, incident data are not shown at all in this chapter.

Chapter 11 (Feeds) and Chapter 12 (Antimicrobial Susceptibility) show only the number of isolations and cultures, respectively.
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 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).

In contrast to *Salmonella* in humans, many isolations of *Salmonella* from livestock are not associated with clinical disease or occur on farm premises where *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’ 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.

The first such report of any particular serovar or serovar and phage 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 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.

Since 2006, any poultry hatchery isolates for which there are no supply flock details available have been treated as isolations only as they cannot be traced back to a specific flock.
The concept of an ‘incident’ is 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*.

All isolates (except some feed and food isolates) that have been identified in England and Wales are required to be sent to an APHA Laboratory for examination and confirmation of *Salmonella*. Of those samples taken in Scotland, the majority of poultry samples are sent to APHA Lasswade and all mammalian samples are sent to the SAC Consulting (part of SRUC) and confirmed by Health Protection Scotland.

Data from research projects and surveys are excluded from the tables in the species chapters in this publication. The antimicrobial susceptibility chapter (Chapter 12) contains data from routine surveillance and other surveillance projects.

**SEROTYPING AND PHAGE TYPING METHODS**

*Salmonella* isolated from animals and feed is biochemically or serologically confirmed and serotyped by micro, tube and/ or slide agglutination tests. Each culture is tested for the presence of somatic and flagella antigens by agglutination with specific *Salmonella* antisera. Where homologous antiserum and antigen react, clumps of bacteria form as visible agglutination. Serovars are derived by reference to the White-Kauffmann-Le Minor Scheme. Additional biochemical tests are needed to confirm some serovars. *Salmonella* Typhimurium, *S.* Enteritidis, *S.* Hadar, *S.* Pullorum, *S.* Thompson and *S.* Virchow can be phage typed according to the PHE phage typing schemes; however, since 2010, only *S.* Typhimurium and *S.* Enteritidis have been routinely phage typed due to economic constraints. Cultures are seeded onto special agar plates and a specific set of phages applied to the culture. After incubation, the pattern and degree of lysis is read and a phage type attributed to the culture (Anderson et al 1977, Ward et al 1987). In the case of *S.* Typhimurium, some phage types are not fully validated as being stable and specific for the serovar. These are referred to as undesignated phage types (U) rather than definitive phage types (DT).

Serotyping and phage typing of samples received from premises in England and Wales is carried out by the APHA. Mammalian isolates, and some poultry isolates, from Scotland are serotyped and phage typed by HPS and the majority of poultry samples from Scotland are serotyped and phage typed by APHA.
Some phage types may be ‘related variants’ although they are still reported as distinct types, e.g. PT4 and PT7 of S. Enteritidis and DT12, DT104, DT104b and U302 of S. Typhimurium. More than one phage type may sometimes be recovered from a group of animals that is sampled by means of environmental samples. This may result from variations in the attachment of phages to organisms that have been exposed to environmental stress.

Monophasic or aphasic group B Salmonella strains, which lack one or both sets of flagella antigens, can be confirmed as variants of S. Typhimurium by obtaining a definitive phage type (DT) for the strain and by S. Typhimurium specific PCRs. PCR methods can also be used to confirm the absence of flagella genes (rather than poor expression of flagella proteins) and the presence of a particular genomic island that is characteristic of the recently emerged (since 2006) monophasic S. Typhimurium DT193/120 variant strains.

A new procedure for simplified presumptive identification of Salmonella Derby from certain known persistently infected turkey fattening farm sources was introduced in 2016 to reduce the cost of serotyping large numbers of such isolates. In this new process, slide agglutination testing of such Group B isolates is used to identify S. Derby [4,5,12:f,g;((1,2)], and to exclude the possibility of S. Typhimurium [4,5,12:i:1,2] or S. Agona [4,5,12:f,g,s:1,2]. After confirmation, the isolates are reported as ‘presumptive S. Derby’ as full serotyping has not been carried out. Any non-Group B isolates, isolates which react with Hi or Hs antiserum or isolates for which definitive S. Derby-related antigens cannot be confirmed are subjected to full serotyping.

METHODS USED FOR SCREENING SALMONELLA VACCINE STRAINS
Following the introduction of live vaccines for Salmonella Enteritidis and Salmonella Typhimurium in poultry, additional testing is required to distinguish field strains from vaccine strains.

Salmonella Enteritidis and relevant S. Typhimurium isolates are compared to the Avipro Vac E and Vac T vaccine strains, which carry antimicrobial resistance markers, using a panel of four relevant antimicrobials in a disc diffusion technique. 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 additional high level resistance to streptomycin and Vac T has an additional resistance to nalidixic acid. Avipro Duo comprises a mixture of Avipro Vac E and Vac T.
Gallivac (Salmovac) SE vaccine has no resistance markers but contains mutations causing auxotrophism for histidine and adenine. Salmonella 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 antimicrobials is carried out at APHA Weybridge and Lasswade. Confirmatory tests for Vac E and Vac T vaccine strains and Gallivac (Salmovac) SE vaccine tests are carried out at APHA Weybridge.

Vaccine strains are excluded from the text, tables and figures of this report.

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 largely differs from that used for the other five subspecies in that the familiar serovar names are assigned to serovars within subspecies enterica whilst members of the other subspecies are designated by antigenic formulae.

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 Grimont & Weill (2007).

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 (4,12: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.
Similarly, *Salmonella* Pullorum is now designated as *S. Gallinarum* biovar Pullorum and some other individual serovars have also been consolidated as variants of a single serovar (e.g. *S. Orion*/Binza).

The serovar previously reported as *S. Binza* is now recorded under the updated nomenclature of *S. Orion* var. 15+ but during 2009 was reported as *S. Orion* (together with *S. Thomasville* which is now referred to as *S. Orion* var. 15+ 34+). It is for this reason that the tables of this publication show no reports of *S. Binza*.

The serovar previously reported as *S. Newbrunswick* is now recorded under the updated nomenclature of *S. Give* var. 15+.