

# Packed with *Salmonella*—Investigation of an International Outbreak of *Salmonella* Senftenberg Infection Linked to Contamination of Prepacked Basil in 2007

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## Abstract

*Salmonella* Senftenberg is uncommon in the United Kingdom. In January–June 2007, the Health Protection Agency reported on 55 primary human cases of *Salmonella* Senftenberg in England and Wales. In May 2007, fresh basil sold in the United Kingdom was found to be contaminated with *Salmonella* Senftenberg. We launched an investigation to elucidate the cause of this outbreak. Isolates were examined using plasmid profiling and pulsed-field gel electrophoresis, and the outbreak strain (SSFTXB.0014) was identified. We enquired via Enter-net whether other countries had isolated the outbreak strain, analyzed samples of fresh herbs from U.K. retailers, and interviewed patients on food history. Thirty-two patient-cases were referred to this outbreak in England and Wales. Onsets of illness occurred between 5 March and 6 June 2007. Fifty-six percent of patient-cases were females and 90% adults (>20 years old); three were admitted to hospital as a result of *Salmonella* infection. Scotland, Denmark, the Netherlands, and the United States reported on 19 cases of *Salmonella* Senftenberg infection presenting with the outbreak strain since January 2007. Eight samples of prepacked fresh basil imported from Israel tested positive with the same strain. A minority of patients could recall the consumption of basil before illness, and some reported consumption of products where basil was a likely ingredient. Environmental investigations in Israel did not identify the contamination source. Microbiological evidence suggested an association between contamination of fresh basil and the cases of *Salmonella* Senftenberg infection, leading to withdrawal of basil from all potentially affected batches from the U.K. market.

## Introduction

**S**ALMONELLOSIS IS AMONG THE MOST common foodborne bacterial diseases (Todd,

2006). *Salmonella* Senftenberg is not one of the serotypes most commonly associated with human infection worldwide (Galanis *et al.*, 2006) but has caused infections mainly in Africa

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(Ramadan *et al.*, 1992) and India (Joseph *et al.*, 1990; L'Ecuyer *et al.*, 1996). Similarly, *Salmonella* Senftenberg is not a common serotype from cases of human infection in England and Wales. In 2006, 55 human cases were reported on by the Health Protection Agency (HPA) Laboratory of Enteric Pathogens (LEP). Few outbreaks of *Salmonella* Senftenberg infection have occurred in England and Wales, those documented being associated with infant formula (Sanford *et al.*, 1969; Rushdy *et al.*, 1998). The organism has also been isolated in England and Wales from coconut products (Rushdy *et al.*, 1998), animal feed (Hobbs and Hugh-Jones, 1969; Hugh-Jones, 1969), and livestock (Liebana *et al.*, 2001).

Between January and June 2007, the LEP reported on 55 primary human isolates of *Salmonella* Senftenberg in England and Wales; a significant rise compared to less than 10 in the same time periods in 2006 and 2005. Forty (73%) isolates were received since 9 April 2007 (week 15). Outbreaks of salmonellosis linked to contamination of fresh vegetables have occurred before in England and Wales (Long *et al.*, 2002; Ward *et al.*, 2002; Horby *et al.*, 2003; Sagoo *et al.*, 2003) and elsewhere (Hedberg *et al.*, 1999; Campbell *et al.*, 2001; Sivapalasingam *et al.*, 2004; Takkinen *et al.*, 2005).

We investigated the causes of this outbreak to guide the implementation of control measures nationally and internationally.

## Materials and Methods

### Microbiological investigations

**Strain characterization.** The LEP confirmed all presumptive isolates of *Salmonella* Senftenberg. All isolates were examined using plasmid profiling and pulsed-field gel electrophoresis (PFGE) to identify a possibly distinct outbreak strain, distinguishable from background *Salmonella* Senftenberg infections. Isolates from patients and food were identified as *Salmonella* Senftenberg in accordance with the Kauffman-White scheme (Popoff and Le Minor, 2001; Bale *et al.*, 2007). All isolates were tested for resistance to antimicrobial drugs in accordance with the method of Frost (1994). Isolates were characterized by PFGE (Gatto *et al.*, 2006), and the profile types designated in accordance with the Salm-gene scheme of nomenclature (Peters *et al.*,

2003). Plasmids were extracted by the method of Kado and Liu (1981) and run for 3.5 h at 110 V on 0.7% agarose before staining and visualization.

**Food product testing.** Samples of fresh herbs were collected from U.K. retailers and analyzed for *Salmonella* spp. as part of a national study that started in May 2007 on the microbiological quality of retail fresh herbs (HPA, 2007b). This study was coordinated by the HPA Centre for Infections (CfI), the HPA Regional Microbiology Network (RMN), and the Local Authorities Coordinators of Regulatory Services (LACORS).

### U.K. outbreak investigation

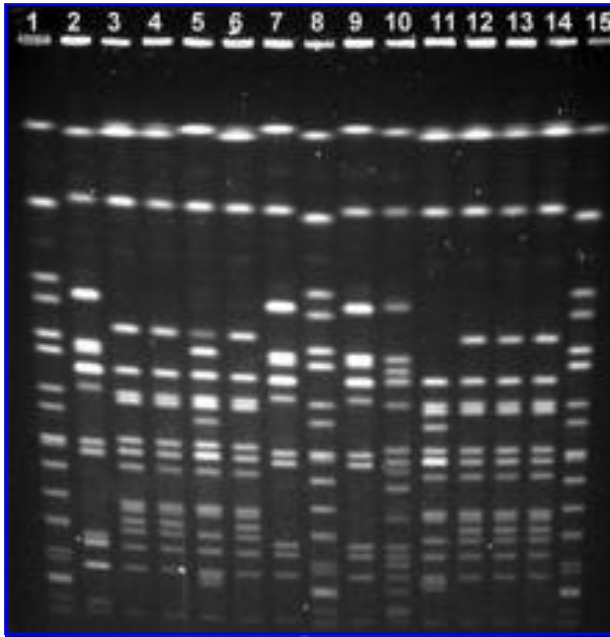
**Case definition.** A case was defined as a resident of England and Wales infected with a drug-sensitive isolate of *Salmonella* Senftenberg, belonging to the same outbreak strain identified with PFGE and plasmid profiling, and received by LEP on or after the 8 April 2007. We excluded cases reporting foreign travel or close contact with a person with gastrointestinal disease symptoms in the 5 days before disease onset.

Cases were interviewed about the 3 days before onset of illness on general food history and on fresh herb consumption using a trawling questionnaire.

### International investigations

**Enter-net.** An enquiry was sent to Enter-net to determine whether other countries had also observed cases or food products with the same strain of *Salmonella* Senftenberg. Additionally, to enquire if the same problem was being experienced elsewhere, we published preliminary results of this investigation in the *Health Protection Report* (HPA, 2007a) and in *Eurosurveillance Weekly* (Pezzoli *et al.*, 2007). The TIFF of the outbreak strain for conversion into Bionumerics was sent electronically to all Enter-net members on 31 May 2007. In the United States, the Centers for Disease Control (CDC) published the information on the outbreak via PulseNet.

**Human infection.** Following the Enter-net enquiry, all countries reporting the isolation of *Salmonella* Senftenberg conducted local epide-



**FIG. 1.** Pulsed-field gel electrophoresis profiles of *Salmonella* Senftenberg isolates from basil, patients, and environmental samples in England and Wales, 2007. Lanes are numerated 1–15. Lanes 3, 4: basil isolates (profile type, SSFTXB.0014). Lanes 6, 12–14: isolates from human cases from England and Wales. Lanes 2, 5, 7, 9–11: other profile types from isolates of *Salmonella* Senftenberg from patients and environmental samples made in England and Wales in 2007. Lanes 1, 8, 15: H9812 (*Salmonella* Braenderup—PulseNet).

miological and microbiological investigations. Isolates of *Salmonella* Senftenberg identified by these countries in 2007 were analyzed using PFGE. Patients infected with the outbreak strain were interviewed for history of food consumption by public health officials in the reporting countries.

European Rapid Alert System for Food and Feed (RASFF). The U.K. Food Standards Agency (FSA) sent an RASFF to the European Commission (EC) to ascertain whether basil from the same batches was on sale elsewhere in the European Union and to clarify the origin of this product. The RASFF included product traceability details, such as product brand name, product aspect (i.e., prepacked), unit weight, batch number, durability date (i.e., use by date), U.K. importer, and country of origin, necessary to identify the distribution line of the product. Following generation of a RASFF by a member state, an International Food Safety Authorities Network (INFOSAN) message would then be

generated by the EC to the World Health Organization (WHO).

**Environmental investigation.** In Israel, the investigators took samples from the herb growers exporting the basil contaminated with *Salmonella* Senftenberg. Samples from the irrigation water, fields, sewage, herbs from greenhouses, and delivery crates were analyzed for *Salmonella* contamination. Swabs from hands and stools of workers were also examined.

## Results

### Microbiological investigations

**Strain characterization.** A specific strain of *Salmonella* Senftenberg was involved in this outbreak. Isolates of *Salmonella* Senftenberg from basil and patients belonged to the same PFGE profile, designated SSFTXB.0014 (Fig. 1). Isolates were characterized by the possession of four plasmids of approximately 6.3, 4.5, 4.2, and 3.0 kb.

**Food product testing.** During May 2007, the outbreak strain of *Salmonella* Senftenberg was detected in eight samples of intact prepacked fresh basil sold in the United Kingdom and grown in Israel. In addition, prepacked fresh basil purchased from a supermarket in the Shetland Islands, Scotland, which was the subject of a customer complaint, was also found to be contaminated with the same outbreak strain. On 25 May 2007, the U.K. retailers affected withdrew all their potentially affected basil stocks with a best before date up to and including 28 May. The U.K. FSA advised consumers who may have bought the affected batches of basil not to eat them (FSA, 2007a).

### U.K. outbreak investigation

Thirty-two isolations of *Salmonella* Senftenberg fulfilling the case definition were reported from 10 April 2007 to 14 June 2007 from cases of infection in all nine Governmental Regions of England and also Wales (Fig. 2).

One patient, with the same outbreak strain, reporting travel to Morocco, was excluded from the case definition. No patients with history of close contact with a person with

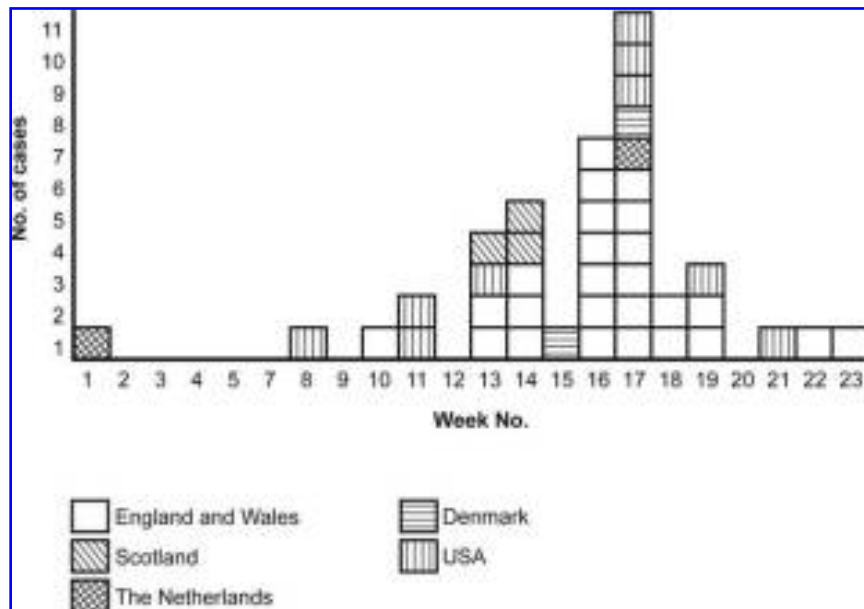


FIG. 2. Epidemic curve of *Salmonella* Senftenberg infections according to available onset dates in England and Wales, Scotland, the Netherlands, Denmark, and United States—January to June 2007;  $n = 41$ .

gastrointestinal disease symptoms were identified and excluded.

Dates of onset of illness were available for 23 cases, ranging from 5 March to 6 June 2007. Of the 32 cases, 18 (56%) were females, while 14 (44%) were males. The median age for case-patients was 48 years, ranging from less than 1 to 93 years. Twenty-nine cases (90%) were >20 years old. Two cases were in the 0–4 age group. Seven cases were admitted to hospital during their illness. Three of these patients were admitted to hospital as a result of *Salmonella* infection; while the other four reported serious underlying illnesses prior to *Salmonella* infection. At the time of the investigation, three of the hospitalized patients were known to have recovered and two of them were interviewed. For one, exposure was likely to have occurred prior to hospital admission, and for the other, the diet was extremely restricted but may have included food purchased outside the hospital. One patient died, but this is not thought to be as a result of salmonellosis.

Twenty of the 32 cases were interviewed. Consumption of poultry, eggs, milk, cheeses, desserts, salads, fruits, and confectionery was reported by >60%. Thirty percent of the interviewees reported having consumed fresh herbs in the 3 days before illness, but only a few could

specifically recall having eaten fresh basil; 40% reported consumption of prepacked leaf salad.

Scotland reported on three cases of *Salmonella* Senftenberg infection in April 2007, all of which shared the outbreak strain (Fig. 1). One of these patients reported travel to Tenerife.

#### International investigations

Enter-net. Colleagues from the Enter-net confirmed that the genotype involved in the outbreak was different from the strain circulating within Europe during the last 2–3 years, and that the outbreak strain had not been identified in the Salm-gene/PulseNet Europe database over this period. The CDC confirmed that since 1999, the outbreak strain was identified only five times in the U.S. PulseNet database of approximately 580 *Salmonella* Senftenberg isolates.

Human infection. Following the Enter-net alert, four non-U.K. countries, Denmark, the Netherlands, Israel, and the United States, reported isolation of *Salmonella* Senftenberg between January and June 2007.

Denmark reported an increase in cases of *Salmonella* Senftenberg human infection, with a total of 11 cases in 2007, compared to none in the first 6 months of 2006. The PFGE profiles for

three of these cases matched those of the U.K. cases. Of these, two reported that their exposure probably occurred whilst visiting the United States and the United Kingdom.

Two cases of *Salmonella* Senftenberg infection were reported in the Netherlands in January 2007 and three in May 2007. Of these five patients, two matched with the outbreak strain cases (Fig. 2). One patient was diagnosed in May and reported eating a mixed pasta salad, possibly mixed with fresh basil in the week before symptom onset. The other one was diagnosed early in January and could not remember eating basil, but reported eating pesto in a restaurant the week before onset of symptoms.

Eleven human isolates of *Salmonella* Senftenberg matching the outbreak PFGE profile had been identified in the United States during the outbreak period. The median age was 43 years with a range from 2 to 75 years. Ten cases (91%) were adults (>20 years old), while only one was in the 0–4 age group. Females accounted for 9 out of 11 (82%) cases. Onset dates were known for 10 patients and ranged from 28 February to 29 May 2007, with a majority of the cases clustering toward late-April/mid-May (Fig. 2). All the patient-cases resided in or had visited the northeastern United States during their incubation periods; none reported international travel. Consumption of basil was confirmed for one case; consumption of fresh herbs was probable for other two. Onset dates for cases who consumed fresh herbs were 25 February, 28 April, and 29 April 2007.

The Israeli Ministry of Health reported on seven *Salmonella* Senftenberg infections between January and June 2007, all of which were not caused by the outbreak strain. In 2005, 7 human *Salmonella* Senftenberg isolates were identified; in 2006, 14 isolates were identified in Israel.

**RASFF.** As the product was prepacked, the label contained sufficient traceability details to identify the grower in Israel. The cut basil was exported in bulk in crates and packaged in plastic packs at the country of destination, where the basil was marketed. Basil from the same batches on sale in the United Kingdom was not found in other member states. The grower also exported to the United States, where a formal traceback for basil distribution was not conducted. It is

unlikely that basil from the same U.K. batches was on sale in the United States.

**Environmental investigations.** *Salmonella* Senftenberg was not detected in samples ( $n = 50$ ) of basil, fresh herbs, and from the environment collected in June 2007 by Israeli authorities from the farms exporting to the United Kingdom. All hand and stool samples of the Israeli workers tested negative for *Salmonella* Senftenberg.

The commercial production of fresh herbs in Israel is generally done in greenhouses. To avoid the restrictions imposed by natural seasonality for these herbs and ensure year-round production, crops at different stages of development are grown. No farm animals were kept close to the greenhouses. The water used for irrigation was municipal water (i.e., treated). The farms were adopting the guidelines for the Good Agricultural Practice of Medicinal and Aromatic Plants (GAP/GWP Subcommittee, 2006).

Investigation of the U.K. packers revealed no *Salmonella* contamination on-site.

## Discussion

By utilizing molecular microbiological techniques, the same strain of *Salmonella* Senftenberg, characterized by PFGE and plasmid profiling, was identified in human isolates and in fresh basil on sale in the United Kingdom and grown in Israel. Although the majority of cases in the United Kingdom occurred after 8 April 2007, since the beginning of 2007, the same outbreak strain was reported by Denmark, the Netherlands, and the United States, as well as in patients who reported traveling to Morocco and Tenerife before onset of symptoms. This strain of *Salmonella* Senftenberg was isolated in the affected countries only a few times before this outbreak. Only a minority of cases interviewed could recall precisely the consumption of basil or fresh herbs before onset of symptoms, but some reported consumption of products where basil was likely to be present.

Environmental investigations in Israel carried out after the withdrawal of product in the United Kingdom did not identify where the contamination of the basil occurred. In Israel, *Salmonella* Senftenberg is not a common serotype. The growers appeared to apply good

hygienic standards; no *Salmonella* Senftenberg was found after sampling the sites and the personnel.

Fresh produce outbreaks are generally characterized by short duration and a wide geographic distribution of cases, which correlate to the centralized production and wide geographic distribution of contaminated product, and may present as a gradual, diffuse, and initially unapparent increase in sporadic cases (Sobel *et al.*, 2002). Contamination of prepacked vegetables with pathogenic microorganisms has happened before, leading in some cases to outbreak occurrence (Hedberg *et al.*, 1999; Sagoo *et al.*, 2003; Todd, 2007).

We uncovered this outbreak, because a study on fresh herbs found *Salmonella* Senftenberg in retail products, and during the same time, the HPA LEP reported on a national increase of *Salmonella* Senftenberg human infection. Basil and human isolates were confirmed to share the same molecular profile, suggesting a likely connection between basil consumption and human infection.

The outbreak strain is rare. International outbreak detection was possible due to sharing of information, including PFGE profiles, among all the countries affected via the PulseNet/Enter-net networks.

The proportion of patients, clearly recalling consumption of basil, was small; a larger proportion of individuals reported that this could have been possible. While fresh herbs like basil are fairly unusual products to be consumed alone, they are frequently used as ingredients in a variety of retail-prepared or homemade ready-to-eat products. Basil could therefore have been added to a food in a way that consumers would not identify it and could have eaten it without knowing.

In England and Wales, all patients interviewed were referred after April 2007. The possible exposure time was consistent with the basil being on sale in the U.K. market. Also in the Netherlands and in the United States, cases with possible history of basil consumption appeared to be clustered toward late-April/early-May.

Denmark recently experienced a combined outbreak of enterotoxigenic *Escherichia coli* and *Salmonella* Anatum, where the likely culprit was imported basil from Israel; although all au-

thorities involved were very cooperative, the traceback proved to be difficult and the exact origin of the basil was never elucidated (Bagdonaite *et al.*, 2006).

During our investigation, the product traceback was effective for withdrawal purposes in the United Kingdom and for identification of the grower. It was even possible to identify exactly from which area of greenhouse the contaminated basil originated. It was impossible to establish precisely to which other countries the growers were exporting fresh herbs and which batches were involved. RASFF does not have mandate to investigate the product chain outside Europe.

We considered the microbiological evidence sufficiently strong to undertake public health actions. We decided not to confirm epidemiologically the link between consumption of basil and illness. As mentioned, in the United Kingdom, FSA issued a public alert regarding contaminated basil to consumers (BBC, 2007; FSA, 2007b). This measure, although a very sensible public health action, was considered to introduce a considerable amount of bias in any analytical study originally planned. In addition the majority of cases were reported to the LEP before this alert was published and only four cases were reported after the 25 of May 2007 in England and Wales, indicating that the outbreak was coming to an end.

## Conclusions

This investigation highlights that fresh herbs sold as prepacked ready-to-eat products can harbor harmful bacteria. Incidents like the one described have occurred previously and may happen again.

The production of ready-to-eat vegetables and herbs on sale in the United Kingdom and elsewhere is closely controlled using the principles of Hazard Analysis and Critical Control Point (HACCP), together with good practice industry guides, which places less emphasis on end product testing. We highlight the role of targeted microbiological food surveillance in identifying food safety incidents thus providing an opportunity to review and inform HACCP procedures.

We were reminded that the use of analytical epidemiology to investigate outbreaks such as

these may be limited by the number of people affected, complex distribution of products through different outlets and/or countries, or the widespread use of a contaminated product as an ingredient in a variety of foods, which inevitably affects recall by patients.

We demonstrated how a combination of food surveillance, molecular microbiology, and epidemiology communicated through established networks allows information to be gathered quickly and disseminated effectively.

We support the increasing use of PulseNet Europe and prompt communication of molecular profiles via the network to facilitate the detection of similar outbreaks in the future.

We also support using specific networks, not only the Enter-net, coordinated by the European Centre for Disease Control (ECDC), or the RASFF, but also the recently introduced International Health Regulations, the INFOSAN, both coordinated by WHO, and the ECDC European Early Warning System, as primary tools for outbreak investigation.

Further, we recommend that RASFF is granted full mandate to investigate product lines outside Europe, if supplied to the European Union market.

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