Salmonella enterica, subspecies enterica, serotype Enteritidis Outbreak at a Lodge in Mokopane, Limpopo Province, January 2014

For the degree: Masters in Public Health (MPH)

Author: Akhona Tshangela

Student number: 13406222

Contact no.: 076 298 4644

E-mail: atshangela@gmail.com, akhonat@nicd.ac.za

Co-supervisors:

Dr Charles Mugero, Division of Public Health Surveillance and Response, NICD-NHLS

Mr Eric Maimela, Limpopo Department of Health and Social Development

Dr Lazarus Kuonza, SAFELTP, NICD-NHLS and University of Pretoria, SHSP
# Table of Contents

Declaration of plagiarism .................................................................i
Dedication ...................................................................................ii
Acknowledgements .....................................................................iii
List of abbreviations ....................................................................iv
Executive Summary ......................................................................v

## Part A: Introduction

- Foodborne Diseases ..................................................................1
  - Salmonella infections .........................................................2
  - *Salmonella enterica*, subspecies enterica, serotype Enteritidis (*Salmonella Enteritidis*) ..................................................5
- Food Safety concerns ..............................................................7
- Background to the outbreak ....................................................8

## Part B: Journal Article

- Abstract ..................................................................................17
- Introduction ...............................................................................18
- Methods ..................................................................................21
  - Detection of the outbreak ....................................................21
  - Case finding and cohort study .............................................21
  - Environmental Investigation ..............................................22
  - Laboratory investigation ....................................................23
  - Data Analysis ........................................................................23
- Results .....................................................................................24
  - Case findings and cohort study ...........................................24
  - Environmental Findings ......................................................30
  - Laboratory Findings ............................................................32
- Discussion ................................................................................33
- Conclusion ...............................................................................36
- Acknowledgements ..................................................................37
- Disclosure Statement .............................................................37
- References ...............................................................................38

## Part C: Secondary Results

- 2 -
Medical Treatment and Laboratory findings.......................................................... 42
Further evidence of Contamination ...................................................................... 42
  Univariate Analysis ............................................................................................... 42
  Environmental assessment of the in-kitchen at the lodge ...................................... 48
Discussion ............................................................................................................... 51
Recommendations .................................................................................................... 52
References................................................................................................................. 53
Author’s guidelines: Foodborne Pathogens and Disease...................................... 54
Declaration of plagiarism

The following declaration must accompany all written work submitted while you are a student of the School of Health Systems and Public Health (SHSPH). No written work will be accepted unless the declaration has been completed and attached.

Declaration

1. I understand what plagiarism is and I am aware of the University's policy in this regard.

2. I declare that the work hereby submitted is my own original work. Where other people's work has been used (either from a printed source, Internet or any other source), this has been properly acknowledged and referenced in accordance with the SHSPH's requirements.

3. I have not used work previously produced by another student or any other person to hand in as my own.

4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

SIGNATURE: ........................................................................................................................................

DATE: ..................................................................
Dedication

To my beautiful daughter, Amangile Asisipho Tshangela.

You are loved unconditionally.
Acknowledgements

South African Field Epidemiology and Laboratory Training Programme and staff:
For providing resources, support and guidance to complete this project. As well as all
the other projects I have completed in the past two years.

Dr Charles Mugero: For substantial contributions to the conception and design of the
journal article as well as acquisition of data and revising the article critically for
important intellectual content.

Eric Maimela: For contributions to conception and acquisition of data as well as
revising the journal article critically for important intellectual content.

Dr Lazarus Kuonza: For contributions to analysis and interpretation of data; and
revising the journal article critically for important intellectual content.
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CEDb</td>
<td>Centre for Enteric Diseases, Bacteriology Division</td>
</tr>
<tr>
<td>CEO</td>
<td>Chief Executive Officer</td>
</tr>
<tr>
<td>CEZD</td>
<td>Centre for Emerging and Zoonotic Diseases</td>
</tr>
<tr>
<td>CIF</td>
<td>Case Investigation Form</td>
</tr>
<tr>
<td>EA</td>
<td>Environmental assessment</td>
</tr>
<tr>
<td>FET</td>
<td>Further Education and Training</td>
</tr>
<tr>
<td>FBD</td>
<td>Foodborne diseases</td>
</tr>
<tr>
<td>MCS</td>
<td>Microscopy, culture and sensitivity</td>
</tr>
<tr>
<td>MPH</td>
<td>Masters in Public Health</td>
</tr>
<tr>
<td>NICD</td>
<td>National Institute for Communicable Disease</td>
</tr>
<tr>
<td>NHLS</td>
<td>National Health Laboratory Services</td>
</tr>
<tr>
<td>ORT</td>
<td>Outbreak Response Team</td>
</tr>
<tr>
<td>ORU</td>
<td>Outbreak Response Unit</td>
</tr>
<tr>
<td>SAFELTP</td>
<td>South African Field Epidemiology and Laboratory Training</td>
</tr>
<tr>
<td>spp</td>
<td>Species</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
Executive Summary

Foodborne disease (FBD) has emerged as a growing public health concern worldwide. Non-typhoidal *Salmonella* spp. cause an estimated 1.4 million cases of FBD in the United States alone each year. *Salmonella enterica*, subspecies enterica, serotype Enteritidis (*Salmonella Enteritidis*) is one of the most commonly reported NTS serotypes associated with FBD worldwide. Since the first poultry-associated outbreak in 1991, in humans, the incidence of *Salmonella* Enteritidis in South Africa has increased. In a study conducted on samples collected between December 2002 and March 2003, *Salmonella* Enteritidis was the second most common serotype to be isolated. Although it is not feasible to reduce all risk for all foods, food industry and risk assessment managers need to identify the risks that have the largest impact on public health. Food safety and risk assessment needs to play an integral role in containing foodborne outbreaks.

On 20 January 2014, a group of 49 nurses, and two training facilitators from the District Department of Health checked into a lodge in Mokopane, Limpopo Province to attend a training workshop. They found 62 college students who had already been staying at the lodge since October 2013. Some of the lodgers started to feel ill shortly after midnight on 22 January 2014. They were taken to the nearest hospital for management. This incident was reported to the Limpopo Provincial Department of Health (LPDoH) Epidemiology Office on the same Friday, 24 January 2014. An outbreak response team (ORT) was assembled and activated to respond to the suspected FBD outbreak. A National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Services (NHLS), Outbreak Response Unit (ORU) staff member and a South African Field Epidemiology and Laboratory Training Programme (SAFELTP) resident joined the ORT on 28 January 2014.

During the outbreak investigation, a line listing was created to capture epidemiological, clinical and laboratory information on all the lodgers that were interviewed during the outbreak. Laboratory and environmental investigations were also conducted.
A retrospective cohort analysis was used to determine the risks of illness associated with consuming foods and/or beverages at the lodge. The at-risk population were contacted to complete a standard questionnaire related to food and beverages consumed at the lodge, symptoms of illness, visits to healthcare facilities and specimen submission for pathogen testing. Food and water samples were tested, as well as completion of an environmental assessment questionnaire by staff and external caterers. The data was categorised and STATA version 12 was used for multivariable analyses.

A total of 73 ill persons, including 3 laboratory-confirmed infections, were identified: 69/109 (63%) of the selected cohort were seen at health facilities. Of the at-risk population 87% (109/124) completed the standard questionnaire: 66 cases of gastrointestinal illness and 43 healthy individuals were identified, with a corresponding attack rate of 61%. Most of the cases were females (86%, n=57) with a mean age of 33 years (S.D=7.1), and 36% (n=24) of the cases were hospitalised. Epidemiological data suggested a point source outbreak with no further transmission. Statistical analysis of survey data indicated consumption of diluted fruit juice (from concentrate) adjusted by other food and beverage items, presented a risk ratio of 1.5 (95% CI, 1.1-1.8, p=0.032). Environmental analysis indicated increased risks for cross-contamination. Guidelines on food hygiene and safety were not in use. Training of staff as well as supervision of operations in the kitchen was insufficient.

The outbreak was possibly due to contamination of food/beverages prepared in the lodge kitchen, and fruit juice consumption was the main exposure associated with ill cases. Feedback on food safety and hygiene practices to prevent contamination at the kitchen lodge were provided. The importance of utilisation of the food safety guidelines, training of staff prior to recruitment and on-going supervision were emphasised to ensure food hygiene and safety. A tool for regular monitoring of quality of food service at the lodge and similar settings needs to be developed and implemented.
Part A: Introduction

Foodborne Diseases

Foodborne disease (FBD) has emerged as a growing public health concern worldwide (Riemann, 2006; Scallan et al., 2011; Murphree et al., 2012). Due to industrialisation, globalisation (e.g. human migration), mass production of food and the growing demand and utilisation of catering services, the incidence and in some cases the severity of FBD has increased.

In the United States, more than 9 million people each year have FBD caused by 31 major identifiable pathogens. Norovirus was the leading cause of these illnesses (58%), followed by non-typhoidal Salmonella spp. (NTS) (35%). The leading cause of hospitalisation and death was the NTS (Scallan et al., 2011). In South Africa, due to the enhanced disease surveillance systems that were put in place during the 2010 Football World Cup period, the number of FBD outbreaks detected and reported improved (NICD, 2010).

Transmission of the causative agents is not only restricted to consumption of contaminated food and water but could also be faecal-oral as well as through direct contact with the infected host (Riemann, 2006). Susceptibility by host could be due to many reasons such as impairment of the immune system and medication taken by the host which affects the ecology of the gastrointestinal tract (Riemann, 2006).

Outbreak investigations are at the core of identifying and conducting FBD surveillance, therefore the quality of these investigations is important. Most FBD outbreaks reported have an unknown causative agent and food source (Riemann, 2006, Murphree et al., 2012). This could be due to the pathogen being unknown, incorrect sample handling and incorrect laboratory procedures (Riemann, 2006). Therefore epidemiological
methods are needed to understand the distribution and source of the causative agent. When an outbreak is confined by time and space the preferred epidemiological method is a retrospective cohort study. This allows the investigators to compare the people who fell ill and those who did not become ill. This type of investigation further allows investigators to measure the distribution of exposure to the causative agent or source, as well as comparison of disease incidence between exposures (Riemann, 2006).

An evaluation identifying the characteristics that made a successful FBD outbreak investigation (i.e. those that identified an etiologic agent or food vehicle) found that more than 4 stools need to be obtained in combination with analytic studies. Furthermore, outbreaks that lacked exposure were usually associated with food contamination during preparation and service at food service establishments (Murphree et al., 2012).

**Salmonella infections**

*Salmonella* species (spp.) are gram-negative bacilli which are ubiquitous, and may be found in the environment and a wide range of animal hosts, including humans (Galanis et al., 2006; Sanchez-Vargas et al. 2011). With approximately 2.8 billion diarrheal illnesses worldwide each year, approximately 3% of these illnesses are due to *Salmonella* infections (Majowicz et al., 2010).

*Salmonella* spp. nomenclature is constantly evolving, the most recent which was adopted by the Centers for Disease Control (CDC), based on the World Health Organization (WHO) Collaborating Centre, divides *Salmonella* into 3 species, *Salmonella enterica* (*S. enterica*), *Salmonella bongori* (*S. bongori*) and *Salmonella subterranea*. *Salmonella enterica* is further subdivided into six subspecies, of which the focus of this study will be Enterica subspecies and more specifically *Salmonella enterica*, subspecies enterica, serotype Enteritidis (*Salmonella Enteritidis*) (Figure 1) (Brenner et al., 2000).
Figure 1. Phylogenetic tree of *Salmonella* spp. Highlighted is *Salmonella enterica*, subspecies enterica, serotype Enteritidis (Salmonella Enteritidis). ([http://bioweb.uwlax.edu/bio203/s2009/meinhard_jaso/Phylogenetic%20Tree.htm](http://bioweb.uwlax.edu/bio203/s2009/meinhard_jaso/Phylogenetic%20Tree.htm))

The epidemiology of *Salmonella* infections throughout the world has significantly changed in the past century. Sanitation and hygiene have improved, reducing typhoid *Salmonella* (i.e. *S. enterica*, subspecies enterica, serotype *Typhi* and *Paratyphi*, typhoid fever) (Barrow, 2013; Tauxe *et al.*, 1997), but replaced by the emergence of non-typhoid *Salmonella* spp. (NTS) which have a high global human health impact (Riemann, 2006; Majowicz *et al.*, 2010; Sanchez-Vargas *et al.*, 2011). It is estimated that 93.8 million of *Salmonella* infections are due to NTS and of which 80.3 million are FBD each year (Majowicz *et al.*, 2010).

In the United States for example, NTS causes an estimated 1.4 million cases of FBD each year (Galanis *et al.*, 2006; Hedican *et al.*, 2009; CDC, 2013). From 1998 to 2002, NTS was the most common bacterial foodborne outbreak aetiology reported to the CDC Foodborne Disease Outbreak Surveillance System, accounting for 53% of the 128 multistate outbreaks reported (CDC, 2013). NTS are also suspected to be the leading causes of FBD in South Africa, and just like other sub-Saharan African countries it is usually invasive and found in immune-compromised individuals. Data for FBD in South Africa is still poorly captured, with patients normally presenting and recorded as gastroenteritis at health care facilities (GERMS-SA, 2013, NICD, 2014). Non-invasive
NTS has a seasonal prevalence from late summer to early autumn months (November to March) in South Africa (GERMS-SA, 2012).

*Salmonella* spp. invades non-phagocytic cells in the human host. It penetrates through the intestinal epithelial cells by inducing its own uptake through a process which mimics phagocytosis (Sanchez-Vargas *et al.*, 2011). Whether the infection is going to lead to disease depends on the virulence factors of the *Salmonella* spp. strain and the age, genetic factors and environment of the host (van Asten *et al.*, 2005). *Salmonella* spp. can be isolated from sterile sites such as blood, bone marrow aspirates, stool and urine. Although bone marrow culture are the most sensitive (80% sensitivity) for diagnosis of *Salmonella* spp., microbiological identification and serology from blood and stool culture are the common standard for the identification. Stool cultures are only positive in 30-35% of the cases and this is due in part to the intermittent bacterial shedding (Sanchez-Vargas *et al.*, 2011). The increasing prevalence, virulence, adaptability and antimicrobial resistance of *Salmonella* spp. pose a challenge worldwide (Galanis *et al.*, 2006; Riemann, 2006; Sanchez-Vargas *et al.*, 2011; Barrow, 2013).

Although the primary reservoir for Typhoid *Salmonella* are humans transmitted faecally/orally, for NTS it is usually livestock and which is ultimately transmitted to humans via contaminated food and animal products (Hendriksen *et al.*, 2011; Riemann, 2006; Sanchez-Vargas *et al.*, 2011; Barrow, 2013). Humans are susceptible to infection with NTS, and certain host characteristics render specific groups of people at higher risk for invasive NTS disease and increased morbidity and mortality as a result. Infections are associated with different clinical syndromes and vary in severity. Gastroenteritis and bacteraemia/septicaemia are the most common along with endovascular infection and focal infection (Sanchez-Vargas *et al.*, 2011; Riemann, 2006). Antimicrobial treatment is rarely considered the first line of treatment unless there are underlying illness and the most at risk group for invasive disease (Sanchez-Vargas *et al.*, 2011). In such cases fluoroquinolone is the first line of treatment.
While in high income countries NTS commonly presents as gastroenteritis, in sub-Saharan Africa NTS are the most common cause of community acquired bacterimia (MacLennan et al., 2013). This is mainly found in immune-compromised people due to the high prevalence of HIV in these countries (Gordon et al., 2008, Paglietti et al., 2013).

*Salmonella* Enteritidis and *Salmonella enterica*, subspecies enterica, serotype Typhimurium are particularly the major causes of FBD (Riemann, 2006; Sanchez-Vargas et al., 2011; Barrow, 2013). These two serotypes were the most frequently isolated from humans worldwide in most regions from 2001 to 2007 (Hendriksen et al., 2011). They are also documented in many multi-state and national outbreaks (Braden et al., 2006; Jain et al, 2009). This study reports on an outbreak caused by *Salmonella* Enteritidis.

*Salmonella enterica*, subspecies enterica, serotype Enteritidis (*Salmonella Enteritidis*)

During the period of 2000 to 2002, *Salmonella* Enteritidis was the most common serotype reported from human isolates globally, accounting for 65% of all human isolates in the WHO Global Salmonella-Surveillance database (Galanis et al., 2006). According to the database, *Salmonella* Enteritidis was the most common *Salmonella* spp. serotype reported in European countries (accounting for 79% to 84% of the isolates between 2000 and 2002) and in Africa, *Salmonella* Enteritidis accounted for 26% of human isolates (Galanis et al., 2006; Janmohamed et al., 2011). Infections have decreased over the decade, with a proportion decrease from 73.9% in 2001 to 55% in 2007 (Hendriksen et al., 2011).

In the US, of the NTS outbreaks reported between 1998 and 2008, 10% were *Salmonella* Enteritidis. In South Africa, since the first poultry-associated outbreak in 1991, in humans, in the Western Cape Province, the incidence of *Salmonella* Enteritidis has increased (Mare et al., 2001; Kruger et al., 2004; GERMS-SA, 2012). In a study conducted
on samples collected between December 2002 and March 2003, *Salmonella* Enteritidis was the second most common serotype to be isolated [4]. Through the Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA) surveillance programme, *Salmonella* Enteritidis has been identified as the most common NTS isolated from humans and causing invasive disease (GERMS-SA, 2013).

The first laboratory confirmed outbreak of foodborne *Salmonella* Enteritidis, was identified by Gartner in 1888, “Gartner-bacillus”. Fifty-eight people, from 25 different families had eaten beef and developed acute gastroenteritis and one fatality. Gartner isolated the bacillus from the infected cow and organs of the fatal case (Riemann, 2006).

A person who has been infected with *Salmonella* Enteritidis usually presents with fever, abdominal cramps, vomiting and diarrhoea beginning 6 to 72 hours after eating the contaminated food or beverage. The severity of illness is usually dose-dependent, higher doses seem to provoke a more intense gastrointestinal response and earlier onset of symptoms (Mintz et al., 1994). The illness can last 4 to 7 days and most persons recover without antibiotic treatment, and may not even seek medical care, but the diarrhoea maybe severe enough to necessitate hospitalisation (Patrick et al., 2004).

The main source of *Salmonella* Enteritidis is contaminated food, predominantly raw eggs, egg products and poultry (Hennessy et al., 1996; Patrick et al., 2004; Janmohamed et al., 2011; Moffat et al., 2013; CDC, 2013; Han et al., 2013). Other food commodities that have also been identified as the source of *Salmonella* Enteritidis infection include pork, beef, raw vegetables, and unpasteurised milk or juice (Patrick et al., 2004).
Food Safety concerns

Even before the identification of the infectious agents, FBD has long been known to exist. The identification of foodborne parasites in the 1800s was a great advance in scientific methods, which paved the way to the modern food safety (e.g. meat inspection) methods (Riemann, 2006).

In the US, food prepared in a restaurant accounted for 54% of the reported outbreaks between 1998 and 2008, and 20% were associated with food prepared in private homes (CDC, 2013). Infected food-handlers have also been implicated as source of foodborne outbreaks of Salmonella spp. in multiple investigations (Beatty et al., 2002; Medus et al., 2006). Food safety and risk assessment therefore needs to play an integral part in containing these outbreaks. Although it is not feasible to reduce all risk for all foods, food industry and risk assessment managers need to identify the risks that have the largest impact on public health (Barrow, 2006). For example, a study identified that most FBD were attributable to plant commodities, with leafy vegetables having the most illnesses and second most frequent hospitalisations. Most deaths were attributable to land animal commodities, specifically to poultry. A clear indication that efforts were needed to prevent contamination of produce and poultry. Outbreaks of Salmonella spp. infections transmitted by tomatoes, juice and sprouts link to underlying concerns about contamination of foods consumed raw (Painter et al., 2013).

In 2010, the Food and Drug administration (FDA) passed the egg safety rule designed to reduce contamination of shell eggs with Salmonella Enteritidis. Furthermore, the Food Safety Moderation Act of 2011 requires the CDC to strengthen surveillance and outbreak response as well as give FDA additional authority to improve food safety (CDC, 2013). South Africa has legislations in place to prevent contamination of food from occurring; however food establishments remain the most vulnerable to FBD outbreaks and where the majority of cases are identified. For example the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972) are in effect to prevent food
contamination; however these are rarely implemented. There is also the Consumer Protection Act 68 of 2008 to protect consumers against any damages, such as medical costs, incurred from goods received (food or beverage consumed in this case) from suppliers (food establishment). However, such legislation is rarely enforced in food establishments.

**Background to the outbreak**

On 20 January 2014, a group of 49 nurses, and 2 facilitators, from the Waterberg District of the Limpopo Province, checked into a lodge in Mokopane town, for a workshop. They found 62 college students who had already been staying at the lodge since October 2013. The lodge’s in-house kitchen provided the students with breakfast, lunch and dinner each day. The nurses ate breakfast and dinner with the students, but in addition the nurses had an external caterer that provided them with tea and lunch during their workshop. On 22 of January 2014, at about midnight, the first group of lodgers complained of feeling ill and were transferred to the nearest hospital (Voortrekker hospital) at about 01:00 am. In total, 32 lodgers were assessed on that day, 29 of whom were discharged on treatment later the same day.

The Voortrekker Hospital CEO, who had been on leave, arrived back at the hospital on 24 January 2014. On that day, three of the case-patients admitted on 22 January 2014 were still hospitalised, and four new case-patients had subsequently been admitted. He reported this to the Limpopo Department of Health (LPDoH), Epidemiology Office the same day. An outbreak response team (ORT) comprising provisional and district epidemiologists, environmental health practitioners, health educators and hospital administrators was assembled and activated on 24 January to respond to the reported suspected FBD outbreak.

The Outbreak Response Unit (ORU) (Division of Public Health Surveillance and Response, National Institute for Communicable Diseases (NICD)) was notified about the
suspected FBD outbreak on 27 January 2014. An ORU staff member and a South African Field Epidemiology and Laboratory Training Programme (SAFELTP) resident joined the provincial team on Tuesday, 28th of January 2014 to support the outbreak investigation and control activities in the province. The aims of the investigation were to establish the existence and magnitude of the outbreak, determine the possible source and risk factors for the transmission of the causative agent(s), and to institute control and preventive measures.

**Problem statement**

Limited literature is available in South Africa capturing FBD data, as well as identifying the major players in FBD (NICD, 2014). Epidemiological investigations are required to establish factors such as the causative agents, mode of transmission and risk factors relating to FBD; in especially food establishments were individuals are most at risk. Some of these aspects are usually lacking in FBD investigations and thus compromising the implementation of target resource efficient interventions and preventative measures.

Estimates of the overall number of episodes of FBD are needed. However, there are challenges in obtaining the true estimates. Factors that contribute include under or lack of reporting of episodes as well as late reporting of FBD outbreaks by health facilities. Therefore, literature needs to be pooled together in other estimate the true burden of this pathogen. Another public health concern is the lack of adherence to food safety and hygiene guidelines in food service establishments.
Purpose of the study

The aim of the study was to describe the epidemiological, clinical, laboratory and environmental characteristics of the outbreak using the data that was collected during the outbreak investigation. Based on the lessons learnt, we recommended preventive and long-term control measures for FBD outbreaks.

Research question

Do the cases of diarrhoea reported at Mokopane lodge during the period 22 - 27 January 2014 constitute an outbreak, what are the possible risk factors for transmission of causative organism and measures for control?
References


Braden CR. *Salmonella enterica* Serotype Enteritidis and Eggs: A national epidemic in the United States. CID 2006;43:512–517


Riemann HP & Cliver DO (eds) 2006, Foodborne Infections and Intoxications, 3rd edition, United Kingdom.


van Asten AJAM, van Dijk JE. Distribution of “classic” virulence factors among *Salmonella* spp. FEMS Immunology and Medical Microbiology 2005;44:251–259.

**Figures**

Meinhardt J. Salmonella enteritidis…it’s what’s for dinner  website.  
Part B: Journal Article
Salmonella enterica, subspecies enterica, serotype Enteritidis
Outbreak at a Lodge in Mokopane, Limpopo Province, South Africa, January 2014

Running title: Foodborne outbreak: A retrospective cohort study

Akhona Tshangela*1,2, Charles Mugero1,3, Eric Maimela4, Juno Thomas3, Lazarus Kuonza1,2

1South African Field Epidemiology and Laboratory Training (SAFELTP) Programme, National Institute for Communicable Diseases, National Health Laboratory Service, South Africa
2School of Health Systems and Public Health, Faculty of Health Sciences, University of Pretoria, South Africa
3Division of Public Health Surveillance and Response, National Institute for Communicable Diseases, National Health Laboratory Service, South Africa
4Epidemiology Unit, Department of Health, Limpopo, Polokwane, South Africa

*Corresponding author contact details:
Address: No. 1 Modderfontein Road, Sandringham, Johannesburg, South Africa.
Email: atshangela@gmail.com
Contact number: +27 11 386 6542
Abstract

**Background:** *Salmonella* Enteritidis is a leading cause of foodborne disease worldwide, but there is little data available in South Africa. The strict observation of food handling regulations is critical in ensuring food hygiene and safety to minimise foodborne outbreaks. We investigated the aetiology of an acute gastroenteritis outbreak among persons staying at a lodge in Limpopo Province, South Africa in January 2014.

**Methods:** A retrospective cohort analysis was used to determine the risks of illness associated with consuming foods and/or beverages at the lodge. The at-risk population were contacted to complete a standard questionnaire related to food and beverages consumed at the lodge, symptoms of illness, visits to healthcare facilities and specimen submission for pathogen testing. Food and water samples were tested, as well as completion of an environmental assessment questionnaire by staff and external caterers. The data was categorised and STATA version 12 was used for multivariate analyses.

**Results:** A total of 73 ill persons, including 3 laboratory-confirmed infections, were identified: 69/109 (63%) of the selected cohort were seen at health facilities. Of the at-risk population 87% (109/124) completed the standard questionnaire: 66 cases of gastrointestinal illness and 43 healthy individuals were identified, with a corresponding attack rate of 61%. Most of the cases were females (86%, n=57) with a mean age of 33 years (S.D=7.1), and 36% (n=24) of the cases were hospitalised. Epidemiological data suggested a point source outbreak with no further transmission. Statistical analysis of survey data indicated consumption of diluted fruit juice (from concentrate) adjusted by other food and beverage items, presented a risk ratio of 1.5 (95% CI, 1.1-1.8, p=0.032). Environmental analysis indicated increased risks for contamination.

**Conclusion:** The outbreak was possibly due to contamination of food/beverages prepared in the lodge kitchen, and fruit juice consumption was the main exposure associated with ill cases. Feedback on food safety and hygiene practices to prevent contamination at the kitchen lodge were provided.
Introduction

Foodborne diseases (FBD) have emerged as a growing public health concern worldwide (Riemann, 2006; Scallan et al., 2011; Murphree et al., 2012). Due to industrialisation, globalisation (e.g. human migration) and mass production of food produce, the incidence and severity of FBD has increased. Most foodborne illnesses can be prevented by decreasing contamination of some foods and reducing illness caused by pathogens.

Salmonella species (spp.) are a common cause of FBD worldwide (Galanis et al., 2006) and have a widespread distribution in the environment. Certain host factors make humans particularly susceptible to infection (Poppe, 1999). *Salmonella* spp. are increasingly becoming antimicrobial resistance; and the prevalence, virulence, and adaptability of this species are a challenge (Poppe, 1999). *Salmonella* spp. causes an estimated 1.4 million cases of FBD in the United States each year (Galanis et al., 2006; Hedican et al., 2009; CDC, 2013). This pathogen also has an incidence of 16 per 100,000 population and the third leading cause of hospitalizations in the elderly age group, ≥ 65 years (CDC, 2013). Non-typhoidal *Salmonella* (NTS) *enterica* spp. accounts for a large burden of the disease. In South Africa, NTS are the leading causes of FBD especially in immune-compromised individuals (e.g. HIV), which is due to recurring invasion. Non-invasive NTS has a seasonal prevalence from late summer to early autumn months in South Africa (GERMS-SA, 2012). Since the strengthening of awareness and reporting mechanism of FBD in South Africa in 2010, with ten FBDs reported in the month of June 2010 alone, the numbers of cases reported have increased (NICD, 2010).

*Salmonela enterica*, subspecies enterica, serotype Enteritidis (*Salmonella Enteritidis*) is the most common FBD related bacterial infection reported worldwide (Poppe, 1999; CDC, 2007; Beatty et al., 2009; Suhana et al., 2010). During the period from 2000 to 2002, *Salmonella* Enteritidis was the most common serotype reported from human isolates globally, accounting for 65% of all human isolates in the WHO Global Salm-Surv database (Galanis et al., 2006; Beatty et al., 2009). According to the database, *Salmonella* Enteritidis was the most common serotype reported in European countries (accounting for 79% to 84% of the isolates between 2000 and 2002) (CDC, 2007; CDC, 2008). In Africa, *Salmonella* Enteritidis accounted for 26% of human isolates (CDC, 2013). It is a
zoonotic disease and is usually found in the animal host and can be easily transmitted through food processing and faecal matter in areas of poor sanitation (Poppe, 1999; Daly et al., 2010).

Since the first reported poultry-associated outbreak in humans in 1991 in the Western Cape Province, the incidence of *Salmonella* Enteritidis in South Africa has increased (Mare et al., 2000; Kruger et al., 2004). In a study conducted on samples collected between December 2002 and March 2003, *Salmonella* Enteritidis was the second most common serotype to be isolated (NICD, 2010). Through the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) surveillance programme, *Salmonella* Enteritidis has been identified as one of the most common NTS isolated from humans (GERMS, 2012; Kruger et al., 2004). Although foodborne outbreaks in South Africa are common, outbreak investigations are not routinely performed and there is very little literature published (Niehaus et al., 2011).

In the USA, of the NTS outbreaks reported between 1998 and 2008, 10% were *Salmonella* Enteritidis. Food prepared in a restaurant accounted for 54% of these outbreaks, while 20% were associated with food prepared in private homes (CDC, 2013). Infected food-handlers have also been implicated as a source of foodborne outbreaks of Salmonellosis in multiple investigations (Medus et al., 2006; Beatty et al., 2009). Food safety and risk assessment therefore needs to play an integral part in containing these outbreaks. In 2010, the Food and Drug Administration (FDA) passed the egg safety rule designed to reduce contamination of shell eggs with *Salmonella* Enteritidis. Furthermore, the Food Safety Moderation Act of 2011 requires the CDC to strengthen surveillance and outbreak response as well as give FDA additional authority to improve food safety (CDC, 2013).

The Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972) is in effect to prevent food contamination in South Africa. There is also the Consumer Protection Act 68 of 2008 to protect consumers against any damages, such as medical costs, incurred from goods received (food or beverage consumed in this case) from suppliers (food establishment). However, such legislation is rarely enforced in food establishments.
This paper focuses on an outbreak of *Salmonella* Enteritidis infection at a lodge in Mokopane, Limpopo Province, South Africa. The main objectives of the study were to describe the epidemiological, clinical, laboratory and environmental characteristics of the outbreak using the data that was collected during the investigation, and to recommend preventive and long term control measures.
Methods

Detection of the outbreak

On 20 January 2014, a group of 49 nurses and two training facilitators from the District Department of Health checked into a lodge in Mokopane, Limpopo Province to attend a training workshop. Sixty-two college students were also staying at the lodge for a three month study period using the lodge as their place of residence while at school. On 22nd January some individuals from both groups complained of gastroenteritis illness shortly after midnight. These individuals were then taken to the nearest hospital. The hospital reported the incident to the Limpopo Provincial Department of Health (LPDoH) Epidemiology Office on 24 January 2014. An outbreak response team (ORT) responded to the outbreak on the same day.

Case finding and cohort study

Suspected, probable and confirmed case definitions were developed. A suspected case of gastroenteritis was any person who resided or attended a training workshop event at the lodge from 20-22 January 2014; had an episode of diarrhoea or vomiting or abdominal cramps and/or fever within 7 days of consuming food/beverages at the lodge. A probable case was any person presenting with the abovementioned signs and symptoms, epidemiologically linked to the people residing at the lodge and having consumed food/beverage at the lodge. Any person with the same signs and symptoms within 7 days of consuming food at the lodge, with a laboratory confirmation of Salmonella, was regarded as a confirmed case.

We conducted a retrospective cohort study to test the hypothesis that the gastroenteritis illness was associated with the consumption of a particular food or beverage at the lodge, from 20 to 24 January 2014. All the nurses and students who resided or attended the workshop at the lodge were enrolled into the cohort. In-house kitchen staff was not allowed to eat any of the food provided, however staff that fell ill during the outbreak period were also enrolled in the cohort. The external caterers that
provided for the nurses’ lunch was also excluded unless they fell ill. Persons who arrived at the lodge after the 24\textsuperscript{th} of January were excluded.

Nurses and students were contacted to complete a standard case investigation form (CIF), from 6 days after onset of illness of the first cases. The CIF included a structured menu of more than 60 food and beverage items eaten between 20-22 January. Therefore, the exposure of individuals in the cohort to approximately 60 foods and beverages served at the lodge, during the potential month of exposure period, was determined by the CIF in the structured menu section. The individuals had different meals each service and were asked to select what they ate for a specific meal. They were also asked about any other food consumed not included in the menu as well as any other beverages taken on a specific day. The nurses cancelled the workshop after the first onset of illness and returned to their respective residences. However, after 20 students were sent home for administrative reasons, there were still 42 students residing at the lodge. These students completed the CIF under close supervision. Contact information for the nurses and students no longer at the lodge was obtained and the questionnaire was administered telephonically. The CIF was only administered to the staff and external caterers if they met the case definition.

\textit{Environmental Investigation}

The District Environmental Health Practitioners (EHPs) conducted an inspection of the lodge facilities and food preparation procedures. The external caterer prepared food at her home and therefore it was also visited. Most FBD outbreaks are implicated to food contamination by mixing of food items as well as asymptomatic food handlers. Therefore, environmental assessment forms (EAF) were administered to collect information on hygiene, food preparation and knowledge of health and safety practises of the in-house kitchen cooks and external caterers. Together with the EHPs inspection, this information was collected to establish if the South African food regulations were followed (Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972)) as well as to identify areas in food processing to prevent future outbreaks.
**Laboratory investigation**

Stool samples were collected from eight cases that were admitted in hospital on 22 January 2014. These samples were tested at the hospital laboratory for the detection of foodborne pathogens using standard operating procedures (York and Rodrigues-Wong, 2003). Specimen that had isolated *Salmonella* spp. were sent for further serotyping to the Centre for Enteric Diseases, Bacteriology Division (CEDb), National Institute of Communicable Disease, a division of the South Africa National Health Laboratory Services. There were no leftover food items from the meals eaten during the period of the 20 to 23 January 2014, except for a piece of chicken left from the lunch provided by the external caterer on 21 January. Food samples from 24 January were collected from the lodge. Water samples were also collected from the kitchen at the lodge. These samples were sent to a private laboratory for testing.

**Data Analysis**

Descriptive analysis was used to describe the cohort. Both clinically suspected and laboratory confirmed cases were included in the analysis. Univariable analysis was used to evaluate the attack rates of *Salmonella* Enteritidis and to calculate the risk ratios (RR) with 95% confidence intervals (CI). A multivariable logistic regression model was constructed to adjust for confounding, using food items that had a p-value of less than 0.2 in the univariable analysis. Potential confounders considered in this study consisted of 68 food items. A p-value of less than 0.05 was considered statistically significant. The potential association due to increased risk for contamination from the environment was also analysed. Data were analysed using STATA version 12.1 (Stata Corp, Texas, USA).
Results

Case findings and cohort study

A total of 73 ill persons, including 4 laboratory-confirmed *Salmonella* spp. which lead to 3 laboratory-confirmed *Salmonella* Enteritidis infections, were identified during the outbreak investigation. District health facilities treated 69/124 (56%) of the at-risk population. A total of 109 (96%) of 114 persons in the cohort completed the standard questionnaire. Of the 109 people interviewed, 66 cases of gastrointestinal illness (4 confirmed, 1 probable and 61 suspected) and 43 healthy individuals were identified, with an overall corresponding attack rate of 61% (Table 1). The sex ratio (females to males) was 3.5 for the whole cohort, with a mean age of 33 years (S.D. =7.1). The symptom prevalence for the cases was: abdominal cramps 86%, diarrhoea 81%, fever 61% and vomiting 52%. Twenty three cases (35%) consulted a doctor, 46 (70%) of the cases reported visiting a hospital or clinic, and 17(26%) of the cases consulted a doctor and visited the hospital. There were 24 (36%) hospitalisations and no deaths. Thirteen (20%) of the cases reported not seeking health care. Eleven (17%) of the cases had underlying conditions (2 Diabetes Mellitus type II, 4 Hypertension, 1 Sinusitis, 4 undisclosed): 8 (12%) of these were nurses and 3 (5%) were college students.
**Table 1:** Number of persons affected, *Salmonella enterica*, subspecies enterica, serotype Enteritidis outbreak, Mokopane, Limpopo, South Africa, January 2014.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dates at lodge</th>
<th>Total Ill</th>
<th>Total at-risk population</th>
<th>Laboratory-confirmed infections (SE)</th>
<th>Total Interviewed</th>
<th>Response rate (%)</th>
<th>Attack rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurses</td>
<td>20-22 January</td>
<td>31</td>
<td>51</td>
<td>4 (3)</td>
<td>40</td>
<td>78</td>
<td>78</td>
</tr>
<tr>
<td>Students</td>
<td>October 2013-</td>
<td>34</td>
<td>62</td>
<td>1</td>
<td>58</td>
<td>94</td>
<td>58</td>
</tr>
<tr>
<td>In-kitchen staff b</td>
<td>N/A</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>External caterers b</td>
<td>N/A</td>
<td>0</td>
<td>3</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>66</strong></td>
<td><strong>124</strong></td>
<td><strong>5 c</strong></td>
<td><strong>109</strong></td>
<td><strong>88</strong></td>
<td><strong>61</strong></td>
</tr>
</tbody>
</table>

*a: Ill and met the case definition, not all cases were interviewed
b: Only ill staff and caterers were interviewed
c: 3 laboratory confirmed *Salmonella* Enteritidis, 1 *Salmonella* spp. and 1 *Edwardsiella tarda.*
The median incubation period from the time the implicated food was served was 28 hours (range, 16-92 hours) (Figure 1). The outbreak peaked on 22 January at around midnight and early morning (n=18). The first case on 20 January 2014, early morning, was a nurse who had arrived at the lodge already ill and the last reported date of onset was one case on 24 January in the afternoon.
**Figure 1:** Epidemic curve of cases by date of onset of symptoms, *Salmonella enterica*, subspecies enterica, serotype Enteritidis outbreak, Mokopane, Limpopo, South Africa, January 2014.
More than 25 food items had elevated risk ratios; however four were statistically significant (Table 2). Fruit juice, green beans, mashed potatoes and vegetables consumed on 20 January 2014 were significant risk factors with elevated risk ratios (RR) and therefore had an association with the outbreak. Individuals who drank fruit juice on the day of 20 January were the most at risk of falling ill [RR=1.49; (95%CI, 1.14-1.98); p-value= 0.004]. Forty three people reported drinking the fruit juice on 20 January, with 35 falling ill, an AR of 82%. This included an in-kitchen lodge cook, who did not have any of the other meals. Those who drank water on the day were less likely to get ill [RR=0.68 (95% CI 0.52-0.88; p-value=0.007)]. Coleslaw made of raw cabbage, raw carrots and mayonnaise and consumed during lunch and dinner time on 20 January, also had an elevated RR, but was not statistically significant. Consumption of diluted fruit juice (from concentrate) adjusted by other food and beverage commodities, presented a risk ratio (RR) of 1.5 (95% CI, 1.1-1.8, p=0.032).
Table 2: Exposures associated with illness with a p-value less than 0.05, ranked by risk ratio, *Salmonella enterica*, subspecies enterica, serotype Enteritidis outbreak, Mokopane, Limpopo, South Africa, January 2014.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Total</th>
<th>Cases</th>
<th>AR%</th>
<th>Total</th>
<th>Cases</th>
<th>AR%</th>
<th>Risk ratio (95%CI)</th>
<th>Pexact</th>
<th>Risk ratio (95%CI)</th>
<th>Pexact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit Juice</td>
<td>43</td>
<td>35</td>
<td>81.4</td>
<td>55</td>
<td>30</td>
<td>54.6</td>
<td>1.49 (1.14-1.98)</td>
<td>0.005</td>
<td>1.4 (1.03-1.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Green beans</td>
<td>20</td>
<td>18</td>
<td>90</td>
<td>78</td>
<td>47</td>
<td>60.3</td>
<td>1.49 (1.18-1.88)</td>
<td>0.012</td>
<td>1.1 (0.71-2)</td>
<td>0.62</td>
</tr>
<tr>
<td>Mashed Potatoes</td>
<td>26</td>
<td>22</td>
<td>84.6</td>
<td>72</td>
<td>43</td>
<td>59.7</td>
<td>1.42 (1.10-1.82)</td>
<td>0.021</td>
<td>1.2 (0.77-2)</td>
<td>0.38</td>
</tr>
<tr>
<td>Vegetables</td>
<td>35</td>
<td>28</td>
<td>80</td>
<td>63</td>
<td>37</td>
<td>58.7</td>
<td>1.36 (1.04-1.78)</td>
<td>0.033</td>
<td>1.2 (0.92-1.5)</td>
<td>1.19</td>
</tr>
<tr>
<td>Water</td>
<td>62</td>
<td>35</td>
<td>56.5</td>
<td>36</td>
<td>30</td>
<td>83.3</td>
<td>0.68 (0.52-0.88)</td>
<td>0.007</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
**Environmental Findings**

Investigation by the EHPs of the in-house kitchen found potential for contamination of food. This was also the case at the home of the external caterer. None of the food handlers had ever undergone a medical examination before working in the kitchen. The external caterer and the in-house kitchen staff were given an EAF to complete. Findings from the assessment form indicated food safety and hygiene guidelines were not followed (Table 3). Vegetables and meats were not prepared separately. Approximately 30% stated that the fruit were cleaned before service. Food was also reheated before service, including reheating of leftover food (Table 3). The fruit juice served during breakfast and dinner service was prepared from liquid concentrated juice. Kitchen staff mixed the concentrate with tap water and ice and served it in jugs. The individuals eating the meal poured the juice from jugs into glasses provided from the kitchen. During lunch time individual canned juice from a commercially brand company was served.
Table 3: Environmental assessment of in-kitchen staff (N=8) and external caterer (N=3), Salmonella enterica, subspecies enterica, serotype Enteritidis outbreak, Mokopane, Limpopo, South Africa, January 2014.

<table>
<thead>
<tr>
<th>Sex</th>
<th>N (%)</th>
<th>N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>7 (64)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4 (36)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation</th>
<th>N (%)</th>
<th>N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooks</td>
<td>7 (64)</td>
<td></td>
</tr>
<tr>
<td>Cleaner</td>
<td>1 (9)</td>
<td></td>
</tr>
<tr>
<td>Caterers</td>
<td>3 (27)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Assessment of food hygiene and safety knowledge</th>
<th>Yes (%)</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Availability of hand wash facilities</td>
<td>8(73)</td>
<td>3(27)</td>
</tr>
<tr>
<td>Use of soap</td>
<td>10(91)</td>
<td>1(9)</td>
</tr>
<tr>
<td>Paper towel</td>
<td>3(27)</td>
<td>9(81)</td>
</tr>
<tr>
<td>Personal Protective Equipment (PPE)</td>
<td>8(73)</td>
<td>3(27)</td>
</tr>
<tr>
<td>Maintenance of cold chain</td>
<td>1(9)</td>
<td>10(91)</td>
</tr>
<tr>
<td>Mixing of raw and cooked food</td>
<td>10(91)</td>
<td>1(9)</td>
</tr>
<tr>
<td>Mixing of vegetables and other food stuffs</td>
<td>10(91)</td>
<td>1(9)</td>
</tr>
<tr>
<td>Vegetables prepared separately</td>
<td>1(9)</td>
<td>10(91)</td>
</tr>
<tr>
<td>Meat prepared separately</td>
<td>1(9)</td>
<td>10(91)</td>
</tr>
<tr>
<td>Fruit washed before serving</td>
<td>3(27)</td>
<td>8(73)</td>
</tr>
<tr>
<td>Reheating of leftover food</td>
<td>8(73)</td>
<td>3(27)</td>
</tr>
<tr>
<td>Reheating of food before serving</td>
<td>6(55)</td>
<td>5(46)</td>
</tr>
</tbody>
</table>
Laboratory Findings

Eight stool samples and rectal swabs were obtained from eight nurse cases. *Salmonella spp.* was isolated from four; one of the isolated *Salmonella spp.* was resistant to Amoxicillin. This case was treated with Ciprofloxacin antibiotics. *Edwardsiella tarda* was isolated from one sample. The cultures of *Salmonella* spp. that were sent to the NICD-CEDb laboratory were typed and found to be serotype Enteritidis. Environmental samples which were taken to a private laboratory in Mokopane, were negative for *Salmonella spp.* and no pathogens were isolated.
Discussion

This was a point source outbreak which could have been prevented by following the food regulations that are in place as well as the Hazard analysis and critical control points (HACCP) guidelines. The epidemiological analysis allowed the investigation team to identify the possible source and subsequent transmission of the illness. Although contamination of a number of food commodities was the most likely cause of the outbreak, fruit juice consumed by most of the cases was most significantly associated with the outbreak.

The juice served twice a day at the lodge was prepared from concentrate and diluted with tap water and ice cubes. It is possible that it was contaminated with items that had been in contact with raw meats or unwashed fruits and vegetables at this time. Unfortunately, the fruit juice, ice cubes were not part of the samples taken for laboratory testing. It is possible the fruit juice could have been contaminated with *Salmonella* Enteritidis during processing or dilution. In 2001 in the US, the Food and Drug Administration (FDA) put in place the Juice Hazard Analysis and Critical Control Point (HACCP) systems regulation after various juice products were implicated in significant foodborne outbreaks in the 1990s. *Salmonella* is recognised as a pertinent organism for citrus fruits and *E. coli* O157:H7 and *Cryptosporidium parvum* as pertinent organisms for apple juices. The “pertinent” microorganism is defined as the most resistant microorganism of public health significance that is likely to occur in the juice (Food and Drug Administration).

No previous similar illness was reported from the students that had been staying at the lodge since October 2013. Neither had such illness been reported from other restaurants in the town nor were increases in cases of gastroenteritis illness reported from other areas of the district. The situation was worsened by the fact that monitoring and supervision of kitchen service was rarely done. Therefore there was also a possibility of cross-contamination from another source. The environmental assessment conducted in the lodge’s kitchen revealed a number of weaknesses with regard to food hygiene and safety. Most of the people involved in food preparation had not undergone a medical examination; vegetables and meat dishes were not prepared separately and...
almost a third of kitchen staff believed that fruits were cleaned before service. The use of paper towels for drying hands was limited. The lodge cooks participated in various tasks and roles in the kitchen ranging from cleaning to food preparation and serving. The external caterer being permitted to serve food in the lodge without any guidelines posed a challenge to ensuring food hygiene and safety.

One in-house kitchen staff was also implicated as a possible source of infection. Based on the investigation findings, we can also speculate that this outbreak occurred as a result of an asymptomatic *Salmonella* Enteritidis infection in one employee. In-kitchen lodge staff were not allowed to eat/drink any of the food and beverage commodities at the lodge. However, one of the lodge cooks who became ill on 22 January was the only food-handler who admitted to drinking the fruit juice that day during the preparation process. The food-handler could have been asymptomatic at the time and was the likely source of contamination at the restaurant. There is increasing recognition that food workers are an important source of illness in restaurant outbreaks of *Salmonella* spp.; (Moffat *et al*., 2013). *Salmonella* has the ability to survive on contaminated fingertips and to be transferred from infected fingertips to food (Medus *et al*., 2006; Pether *et al*., 1971). Studies show that infected food-handlers are a primary source of contamination with Norovirus, Hepatitis A virus and Shigella, and that they can also be an important source of contamination with *Salmonella* in commercial food service settings (Medus *et al*., 2006). *Salmonella* spp. has a widespread distribution in the environment and certain host factors make humans particularly susceptible to infection. Some studies have reported shedding of Salmonella for up to 97 days in asymptomatic food-handlers (Medus *et al*., 2006; Pether *et al*., 1971).

The main study limitation was the late reporting of the outbreak that resulted in non-collection of retained food samples for testing. The hospital infection control staff did not report the outbreak to the LPDoH until after 2 days, when the outbreak was identified by the hospital CEO. This was upon his return from leave. Procurement for the lodge was not fully assessed, including the fruit juice supplier, and therefore those facilities were not assessed. Although a blood sample was taken, stool samples from the cook who fell ill during the investigation were not taken. The cook was no longer available for further testing. No pathogens were identified in the blood. Invasive disease
is transient and due to the blood being taken almost two weeks after the outbreak period, this could explain this negative result.

Immediate public health action taken in response to this outbreak to reduce the risk of *Salmonella* transmission included informing in-house kitchen and external caterer about food hygiene and safety. In addition, the lodge management instituted several measures including suspending the in-house kitchen staff that had been on duty during the event, serving only bottled or canned juice/water, and using purified water for cooking. There was no evidence of on-going transmission after these measures were implemented. The outbreak highlighted the gap in ensuring food hygiene and safety, especially in an establishment serving food. The investigation also re-affirmed the importance of understanding guidelines regarding health of employees in the food industry, serving food in restaurants or hotels; system for monitoring food hygiene and safety during food preparation and eating places as well as food suppliers.
Conclusion

This study adds to the growing number of investigations of *Salmonella* Enteritidis outbreak in restaurants globally and possibility of multisource infections. It also implicates an asymptomatic food-handler as the source of the foodborne outbreak (Beatty *et al.*, 2009; Hedican *et al.*, 2009; Medus *et al.*, 2006). Although it is not feasible to reduce all risk for all foods, food industry and risk assessment managers need to identify the risks that have the largest impact on public health (Barrow, 2006). South Africa has legislation in place to prevent contamination of food from occurring, food establishments remain the most vulnerable to FBD outbreaks and where the majority of cases are identified. The implementation of systems for monitoring of food hygiene and safety in eating establishments, including the HACCP system could have public health benefits and reduce the number of foodborne disease outbreaks related to eating places. During foodborne disease outbreak investigations in restaurants, testing of employees, especially food-handlers with or without history of illness is critical as part of comprehensive investigation for the cause and source of infection.

We recommend improvement in the reporting of outbreaks in accordance with the notification system based on government law (National Health Act, Act 61 of 2003). FBD is a notifiable condition in South Africa and all suspected cases need to be reported immediately to ensure rapid response. In order to reduce these outbreaks in food establishments, the enforcement of the South African food regulations is required (Foodstuffs, Cosmetics and Disinfectant Act 54 of 1972). This could be done by development and implementing of monitoring tool to be used by EHPs during routine monitoring of food establishments. Consumers need also to be informed and educated on the Consumer Protection Act, where they have the right to claim damages, such as medical costs, should they be damaged by or due to goods (South African Consumer Act 68 of 2008). The development of a FBD active surveillance network, such as the FoodNet in the US, which is an active population based surveillance. This would be for all laboratory-confirmed infections caused by selected pathogens transmitted commonly through food, to quantify and monitor their incidence. Intersectorial collaborations with the Department of Health as well as Department of Trade and Industry are a necessity to reduce this burden.
Acknowledgements

We would like to acknowledge the EHPs who collected the data. We would also like to thank Dorothy L Southern for scientific writing support in reviewing and editing this paper.

Disclosure Statement

No competing financial interests exist.
References


Part C: Secondary Results
**Medical Treatment and Laboratory findings**

Twenty-four (36.3%) people in the cohort were admitted into hospital. Twelve of the cohort (8 nurses and 4 students) had underlying conditions (i.e. 4 Hypertension, 2 Diabetes Mellitus type II, 1 Sinusitis, 1 Ulcers and 4 unknown). Nine of twelve cases were admitted into hospital. There were 12 admitted cases that could recall some of the treatment administered to them; of these 10 were registered nurses.

Antibiotic treatment was given to 9 of the 12 cases, 8 were treated with Amoxicillin (two had underlying conditions) and one with Ciprofloxacin. Eight stool samples and rectal swabs were obtained from eight nurse cases admitted into hospital. From four of these *Salmonella* spp. was isolated; one of the isolated *Salmonella* spp. was resistant to Amoxicillin which was the case treated with Ciprofloxacin antibiotics. The fifth sample isolated *Edwardsiella* tarda, in the case identified as a diabetic patient.

**Further evidence of Contamination**

**Univariate Analysis**

The exposure of individuals in the cohort to approximately 68 foods and beverages served at the lodge during the potential three days of exposure period was determined using the CIF, in the structured menu section. The individuals had different meals each service and were asked to select what they ate for a specific meal. They were also asked about any other food consumed not included in the menu as well as any other beverages taken on a specific day.

More than 25 food items had elevated risk ratios; however 4 were statistically significant. Fruit juice, green beans, mashed potatoes and vegetables consumed on 20 January 2014 were significant risk factors with elevated risk ratios (RR) suggesting they could be associated with the FBD. Individuals who drank fruit juice on the day of 20 January were the most at risk of falling ill (RR=1.49; (95%CI, 1.14-1.98); p-value=0.004). Forty three people reported drinking the fruit juice on 20 January, with 35 falling ill, an AR of 82%. This included the in-kitchen lodge cook, who did not have any
of the other meals. Water consumed on the same day had protective potential with a RR of 0.68 (95% CI 0.52-0.88; p-value=0.007). Coleslaw, which comprised of raw cabbage, raw carrots and mayonnaise also had elevated RR, consumed during lunch and dinner time of 20 January but was not statistically significant. Results for the multivariate analysis are presented in the journal article.
Table 1: Univariable Analysis of exposures associated with illness, p-value less than 0.05 highlighted in bold, *Salmonella enterica*, subspecies enterica, serotype Enteritidis outbreak, Mokopane, Limpopo, South Africa, January 2014.

<table>
<thead>
<tr>
<th>Consumed during</th>
<th>Food</th>
<th>Number of Persons Who Eaten Specified Food</th>
<th>Number of Persons Who Did Not Eat Specified Food</th>
<th>Risk Ratio</th>
<th>Lower 95% C.I.</th>
<th>Upper 95% C.I.</th>
<th>Chi-squared test Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lunch 20 January</td>
<td>Mashed Potato</td>
<td>22 Ill, 4 Well, Total 26</td>
<td>84.6 Ill, 43 Well, Total 72</td>
<td>1.42</td>
<td>1.103</td>
<td>1.820</td>
<td>5.300</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Green Beans</td>
<td>18 Ill, 2 Well, Total 20</td>
<td>90.0 Ill, 47 Well, Total 78</td>
<td>1.49</td>
<td>1.184</td>
<td>1.884</td>
<td>6.305</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Coleslaw</td>
<td>16 Ill, 4 Well, Total 20</td>
<td>80.0 Ill, 49 Well, Total 78</td>
<td>1.27</td>
<td>0.965</td>
<td>1.681</td>
<td>2.104</td>
<td>0.147</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>15 Ill, 4 Well, Total 19</td>
<td>78.9 Ill, 50 Well, Total 79</td>
<td>1.25</td>
<td>0.937</td>
<td>1.661</td>
<td>1.681</td>
<td>0.195</td>
</tr>
<tr>
<td></td>
<td>Vegetables</td>
<td>28 Ill, 7 Well, Total 35</td>
<td>80.0 Ill, 37 Well, Total 63</td>
<td>1.36</td>
<td>1.045</td>
<td>1.776</td>
<td>4.558</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Coleslaw</td>
<td>9 Ill, 1 Well, Total 10</td>
<td>90.0 Ill, 56 Well, Total 88</td>
<td>1.41</td>
<td>1.090</td>
<td>1.834</td>
<td>2.794</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>Green Salad</td>
<td>13 Ill, 3 Well, Total 16</td>
<td>81.3 Ill, 52 Well, Total 82</td>
<td>1.28</td>
<td>0.961</td>
<td>1.707</td>
<td>1.907</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td>Vegetable Juice</td>
<td>36 Ill, 8 Well, Total 44</td>
<td>81.8 Ill, 30 Well, Total 55</td>
<td>1.50</td>
<td>1.135</td>
<td>1.982</td>
<td>8.182</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>3 Ill, 5 Well, Total 8</td>
<td>37.5 Ill, 62 Well, Total 90</td>
<td>0.54</td>
<td>0.220</td>
<td>1.346</td>
<td>3.241</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>Waffles</td>
<td>5 Ill, 0 Well, Total 5</td>
<td>100.0 Ill, 60 Well, Total 93</td>
<td>1.55</td>
<td>1.333</td>
<td>1.802</td>
<td>2.675</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td>Beef Cocktail Sausages</td>
<td>10 Ill, 1 Well, Total 11</td>
<td>90.9 Ill, 55 Well, Total 87</td>
<td>1.44</td>
<td>1.124</td>
<td>1.839</td>
<td>3.353</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>Cheese</td>
<td>33 Ill, 12 Well, Total 45</td>
<td>73.3 Ill, 32 Well, Total 53</td>
<td>1.21</td>
<td>0.918</td>
<td>1.608</td>
<td>1.829</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td>Juices</td>
<td>37 Ill, 14 Well, Total 51</td>
<td>72.5 Ill, 28 Well, Total 47</td>
<td>1.22</td>
<td>0.911</td>
<td>1.627</td>
<td>1.844</td>
<td>0.175</td>
</tr>
<tr>
<td>Breakfast 21 January</td>
<td>Milk</td>
<td>3 Ill, 5 Well, Total 8</td>
<td>37.5 Ill, 62 Well, Total 90</td>
<td>0.54</td>
<td>0.220</td>
<td>1.346</td>
<td>3.241</td>
<td>0.072</td>
</tr>
<tr>
<td>First reported cases at 12 noon</td>
<td>Lunch 21 January</td>
<td>Boiled spinach</td>
<td>21 Ill, 5 Well, Total 26</td>
<td>80.8 Ill, 44 Well, Total 72</td>
<td>1.32</td>
<td>1.016</td>
<td>1.719</td>
<td>3.305</td>
</tr>
<tr>
<td></td>
<td>Supper 22 January</td>
<td>Coleslaw</td>
<td>14 Ill, 14 Well, Total 28</td>
<td>50.0 Ill, 51 Well, Total 70</td>
<td>0.69</td>
<td>0.461</td>
<td>1.021</td>
<td>4.678</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>35 Ill, 27 Well, Total 62</td>
<td>56.5 Ill, 30 Well, Total 36</td>
<td>0.68</td>
<td>0.521</td>
<td>0.881</td>
<td>7.369</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Environmental assessment of the in-kitchen at the lodge

The refrigerator used to store uncooked foods and raw vegetables had a temperature of 16°C upon inspection by the EHPs (Figure 1). Cooked foods were put together with uncooked foods (Figure 1A) and overall cleanliness of the fridge was questioned (Figure 1B). Personal protective equipment, although available, was not used by the kitchen staff during preparation of meals (Figure 2A). The jugs with left over fruit juice were left next to raw vegetables (Figure 2B).
**Figure 1:** Environmental assessment of the in-kitchen at the lodge. Assessment of the walk-in fridge, *Salmonella enterica*, subspecies enterica, serotype Enteritidis outbreak, Mokopane, Limpopo, South Africa, January 2014. **A**, Cooked foods are put next to dairy products and uncooked chicken livers (indicated by arrows). **B**, Dirty floor in the walk-in fridge.
Figure 2: Environmental assessment of the in-kitchen at the lodge. Assessment of the overall environment of the kitchen, *Salmonella enterica*, subspecies enterica, serotype Enteritidis outbreak, Mokopane, Limpopo, South Africa, January 2014. 

A, No gloves used to prepare fruit salad. 

B, Dirty fruit juice containers next to raw vegetables.
**Discussion**

The first line of treatment for this pathogen in severe cases is fluoroquinolone (Sanchez-Vargas et al., 2011); health workers used Amoxicillin as the first line of treatment for most of the hospitalised cases. Antimicrobial treatment should not usually be considered when treating *Salmonella* Enteritidis (Sanchez-Vargas et al., 2011), however the cases had been hospitalised and antibiotics may have been needed as a route of treatment depending on severity of the symptoms. The high rate of mortality due to invasive *Salmonella* Enteritidis in sub-Saharan Africa also makes antibiotic treatment necessary when treating hospitalised cases (Sanchez-Vargas et al., 2011; Han et al., 2013).

Although the implicated meal was supper time, foods eaten during lunch time of 20 January also had elevated risk ratios; therefore *Salmonella* Enteritidis may have been introduced during this time period. This could imply that the contamination began during the lunch time period, with only the college students being affected. The nurses had an external caterer serving them lunch during their stay. The environmental assessment illustrated that food safety and hygiene guidelines were not followed in this kitchen. Lack of washing of vegetables and fruits in this kitchen may have been the driving factor. Investigators were unable to fully assess whether the coleslaw with the elevated risk on 22 January 2014 had been freshly prepared or left over from the previous meals. The cold chain guidelines were also not followed; chicken livers which should be frozen were found in the standard fridge which had an elevated temperature.

As previously stated, the main source of *Salmonella* Enteritidis is predominantly raw eggs and poultry (Hennessy et al., 1996; Patrick et al., 2004; Janmohamed et al., 2011; Moffat et al., 2013; CDC, 2013; Han et al., 2013). However, other food commodities such as raw vegetables and juice need to be important considerations when conducting FBD outbreaks investigations (Patrick et al., 2004). When food samples are being collected in an outbreak, raw vegetables and juice should be considered. There have been previous findings that indicate that most FBD are attributable to plant commodities, with leafy
vegetables having the most illnesses and second most frequent hospitalisations (Painter \textit{et al.}, 2013). More than a third of the cases were hospitalised in this outbreak.

\textbf{Recommendations}

The importance of implementing guidelines when dealing with FBD outbreaks is further illustrated. Food services operators must implement available guidelines on food hygiene and safety. The provincial CDC Unit should support the district in the development and implementation of forms for supervision and monitoring of eating places. Health professionals must adhere to available guidelines to ensure appropriate assessment of patients and rationale use of medicines in treatment of infections in an outbreak in-particular antibiotic treatment. This is critical in reducing risk of development of multi-drug resistant pathogens.
References


Author’s guidelines: Foodborne Pathogens and Disease