A qualitative risk assessment of *Salmonella enteritidis* in the broiler production chain in the Western Cape, South Africa

By

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Declaration

I hereby declare that this dissertation, which I hereby submit for the Master of Science degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, to be my own work and has not been previously submitted by me for degree purposes at another tertiary institution.

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List of Abbreviations

AMR	Antimicrobial resistance
ASC	Acidified Sodium Chlorite
BGA	Modified brilliant green agar
CCFH	Codex Committee on Food Hygiene
CCP	Critical Control Points
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
FAO	Food and Agriculture Organisation of the United Nations
GLM	General linear model
GMP	Good manufacturing practices
HACCP	Hazard Analysis and Critical Control Points
NTS	Non-typhoidal salmonella
OIE	World Organisation for Animal Health
OR	Odds ratio
PCA	Principle component analysis
PRP	Prerequisite programs
R	Rstudio.
SABS	South African Bureau of Standards
SE	*Salmonella enteritidis*
SNA	Social network analysis
SPS	Sanitary and Phytosanitary Agreement
TSP	Tri Sodium Phosphate
WCDoA	Western Cape Department of Agriculture
WCPVL	Western Cape Provincial Veterinary Laboratory

Summary

A qualitative risk assessment of Salmonella enteritidis in the broiler production chain of the Western Cape South Africa

by

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A risk assessment was conducted for *Salmonella enteritidis* (SE) in the broiler production chain of the Western Cape of South Africa, using a literature review, historical data analysis, microbiological sampling from abattoirs and questionnaire responses.

A questionnaire to characterise the risk pathway in the broiler chain was developed based on a review of the literature.

Spatio-temporal analysis was conducted using historical data of reported SE outbreaks. The data showed spatio-temporal clustering, however, the analysis was not correct for groups of farms that form part of the same organisation.

Neck skin samples were collected from five abattoirs and tested for salmonella. In total, 499 samples were collected, representing 46 broiler farms of which nine (20%) were positive. Salmonella positive cultures (2.81%) were serotyped. No SE was identified during the study.

The questionnaire was completed for all stages of the broiler production chain. Significant risk factors were found using univariate analysis (Odds Ratio,OR). The significant risk factors identified by OR were further analysed using principal component analysis (PCA). The factors moving with or opposing, farm positivity were analysed using logistic regression and one layer farm was found to be a significant factor. The farms receiving eggs or chicks from that layer farm had a number of factors that were found to be significant using OR and therefore the presence of this layer farm could be a representation of these factors at the broiler farm level.

SE was not identified in the samples collected from abattoirs, a number of other non-typhoidal *Salmonella subspecies enterica* were isolated. Given the high rates of immunosuppressive diseases within the South African population, it is important for broiler producers to be aware of the zoonotic potential of non-typhoidal Salmonella and continually improve their control measures.

Chapter 1: **Introduction**

The Western Cape Department of Agriculture (WCDoA) conducts annual food safety audits on all export approved food-processing establishments. During these audits, it was noted that there has been an increase in the detection of *Salmonella spp.* by food processors. In the Western Cape the number of reported cases of *Salmonella enteritidis* (SE) in chickens has been increasing since 2009. In 2009 there were three reported cases, five in 2012, 41 in 2013, 19 in 2015, 12 in 2016 and six in 2017 (van Helden, 2018). These increases were noted without any changes in surveillance methods used for *Salmonella enteritidis* detection in poultry.

The reports originate from the internal surveillance of the farms, hatcheries and abattoirs. Some producers perform their own internal surveillance as part of their hygiene and disease management programs. *Salmonella* detection is often one of many tests conducted. If salmonella is detected from a food/ feed/ equipment or environmental tests, some producers choose to conduct serotyping and if the result is SE, they are legally obliged to report it to the state, however, producers are not legally obliged to serotype salmonella when detected. The same applies if salmonellosis is detected in a chicken.

Salmonella enteritidis is a bacterial pathogen known to be associated with poultry products and is one of the salmonella species responsible for human salmonellosis, which is a significant risk for infants, the elderly and immunodeficient people. The prevalence of SE in poultry of the Western Cape is unknown. A 2013 report from the National Institute of Communicable Diseases described a significant increase in the isolation of SE from human patients in the Western Cape and Gauteng provinces of South Africa over 2011 and 2012 (Keddy, 2013b). In the Western Cape, non-invasive SE isolates increased from 5 per annum in 2005 to a peak of 152 in 2012 and 107 in 2013(Keddy, 2013a). There were no changes in the surveillance system for humans during this time.

According to the Food and Agricultural Organisation's (FAO) guidelines on a value chain approach to animal disease management, must be planned and implemented in proportion to the level of risk associated with the disease (FAO, 2011). The FAO guide explains that different stakeholders (people, groups, organizations) may be affected by and react to disease hazards in different ways, may face, perceive and accept different levels of risk, and that prevention and control measures should be proportionate to the risk faced by each stakeholder (FAO, 2011).

In order to control animal diseases in a planned and proportionate manner two factors must be addressed (FAO, 2011):

1. Understanding the livestock production system, how the stakeholders operate and the decisions they make within the production system,

2. Evaluating disease risks and measures to reduce those risks within the livestock production system.

In a paper published in 1994 on the economic impact of SE infection in humans, Roberts and Sockett described the estimated cost of SE infection for Great Britain in 1992 at between £224 and £321 million and that the largest cost to the economy in Great Britain was production losses (Roberts & Sockett, 1994).

The aim of this study is to analyse the risk of SE in the Western Cape Province of South Africa by describing the risk pathway of SE through the food chain and highlighting the critical points of the chain (where the risk may be introduced or may increase drastically) and to assess how the risk could be mitigated through policy changes, education of food producers and consumers or through implementation of stricter controls.

The assessment will be conducted by examining the controls stipulated in the food safety legislation in South Africa and the control measures that are implemented by Local producers. This assessment will benefit from internationally published studies to determine the points in the food chain where additional control could be most effective.

Three main objectives can be described:

1. Qualitative risk assessment of the SE in poultry in the Western Cape, South Africa
    a. describe from farm to retail the risk pathway of foodborne contamination caused by SE on broiler meat and broiler meat products;
    b. identify target areas along the farm-to- retail continuum for potential risk-reduction activities;
2. Evaluate the qualitative risk assessment as a tool to determine risk in an environment where empirical test data is limited.
3. Gap analysis for future quantitative risk assessments as well as to guide future research and data-collection efforts.

Chapter 2: **Literature Review**

Identification of the hazard for the study is based on literature and the conditions known to control and prevent to the growth of the hazard in the environment are given. The unique transmission strategy of SE is given for understanding assessment of the broiler chain and not only broiler farms. Then the impact the organism and its associated disease has on humans and poultry as a motivation for the study is provided using previously published data. As the study focusses on the production chain and a specific endpoint in the food chain is used for sampling purposes. The summation of controls and risk factors in along the chain are provided. The introduction and transmission of the organism as a pathogen in poultry within the context of the broiler production chain are provided. The fact that SE is a World Animal Health Organisation (OIE) listed disease and as such has an impact on trade is described and the concept of risk analysis with a focus on international standards such as the OIE terrestrial animal health code and Codex Alimentarius commission are briefly described. As SE is also a food safety hazard and risk analysis is a component of many food safety standards with the most common for being HACCP. The concept of HACCP is described and the reason why the form or risk analysis given in HACCP is not adequate in this context is provided. In this section a few useful concepts such as the summation of controls and communication are taken from private standards and introduced to add value to the discussion.

Salmonella enterica subspecies enterica can be divided in the Typhoidal serovars (For example *S. Typhi* and *S. Paratyphi*) and the Non-Typhoidal serovars (for example S. Typhimurium and SE)(Gordon, 2008; de Jong, Parry, van der Poll & Wiersinga, 2012). This study focussed on *Salmonella enterica* subspecies *enterica* serotype *enteritidis* (*Salmonella enteritidis,* SE) which are facultative anaerobic, Gram negative, small rods, they are usually motile with peritrichous flagella (Bell & Kyriakides, 2004). SE can be completely destroyed in eggs in a water bath at 58˚C for 50 to 58 minutes or by immersion in water at 57°C for 65 to 75 minutes (Doyle & Mazzotta, 2000). Limits for growth of salmonella can be found in Table 1(Bell & Kyriakides, 2004).

Table 1: Limits for the growth of Salmonella under otherwise optimal conditions (Bell & Kyriakides, 2004)

Parameter (other conditions being optimal)	**Minimum**	**Maximum**
Temperature (°C)	*5.2*[a]	46.2
PH	*3.8*[b]	9.5
Water activity	0.94	>0.99

[a] Most serotypes will not grow at <7.0 °C.
[b] Most serotypes will not grow below pH 4.5.

2.1. Epidemiology of *Salmonella enteritidis*

In 2010 foodborne diarrhoeal disease agents caused 230,000 of the 420,000 deaths globally due to foodborne hazards. Of these, non-typhoidal *S.enterica* accounted for 59,000, enteropathogenic *Escherichia coli* (EPEC) for 37,000, norovirus for 35,000 and enterotoxigenic *E. coli* (ETEC) for 26,000 deaths. Of the 59,000 global deaths due to non-typhoidal *S. enterica*, 32,000 were in the two African sub-regions (African region D: Algeria; Angola; Benin; Burkina Faso; Cameroon; Cabo Verde; Chad; Comoros; Equatorial Guinea; Gabon; Gambia; Ghana; Guinea; Guinea-Bissau; Liberia; Madagascar; Mali; Mauritania; Mauritius; Niger; Nigeria; Sao Tome and Principe; Senegal; Seychelles; Sierra Leone and Togo. African region E: Botswana; Burundi; Central African Republic; Congo; Côte d'Ivoire; Democratic Republic of the Congo; Eritrea; Ethiopia; Kenya; Lesotho; Malawi; Mozambique; Namibia; Rwanda; South Africa; Swaziland; Uganda; United Republic of Tanzania; Zambia and Zimbabwe), and included 22,000 deaths due to invasive disease by this bacterium (WHO, 2014).

In a retrospective paper that analysed historical data from 1996 to 2006 of samples analysed from South African reference laboratories, 24.8% of the salmonella positive samples in poultry were due to *S. typhimurium* and 9.3 % of the positive samples were due to *S.enteritidis* (Kidanemariam et al., 2010).

As explained in the introduction, non-invasive SE infection in humans has increased in the Western Cape from 2012 and 2013 (Keddy, 2013a) and the cause of this is unknown. It has been shown in other countries that if SE is controlled in the poultry population there is a similar reduction in the incidence of SE in the human population (Foley, Lynne, & Nayak, 2008). We hypothesize that in the inverse sense, an increasing incidence in the human population should be the sign of an increasing incidence in poultry, although this increase in human cases may also be due to other reasons. Therefore, SE is a disease warrants further investigation to gain a better understanding of the epidemiology of the disease.

2.1.1. Impact of *Salmonella enteritidis* infection on poultry

In a review article published by Suzuki in 1994, SE infections in poultry are described as overt and symptomless intestinal infections (Suzuki, 1994). In general, acute outbreaks of SE infection occur in young birds and in birds under extreme stress, and seldom occur in semi-mature and adults under natural conditions (Lister, 1988). In cases of natural infection with SE, a mortality of up to 20% has been observed in chicks less than two weeks of age while a percentage of the chicks that do not die are stunted as a result of infection (Lister, 1988). Infection seldom causes mortality in birds more than one month old (Corrier, Hargis, Hinton, Lindsey, Caldwell, Manning & DeLoach, 1991; Suzuki, 1994). Suzuki cited multiple papers when stating that broiler breeder and layer flocks naturally infected with SE do not show obvious clinical abnormalities, increases in mortality or decreases in egg, although the body condition of some of them was rather poor (Cooper, Nicholas & Bracewell, 1989; Hopper & Mawer, 1988; Humphrey, 1990) and also indicated that some hens were found to be carrying SE in their caeca (Hopper & Mawer, 1988; Lister, 1988). These symptomless carriers may spread the infection in the flock due to contamination of their intestinal contents with SE (Suzuki, 1994).

A review article by Guard-Petter, 2001, described the clinical signs of SE in poultry as silent and cited Snoeyenbos (1991) as saying that eggs contaminated with SE infrequently cause an ovarian granuloma or "Pullorum lesion", which is pathognomonic for evidence of infection associated with internal egg contamination (Guard-petter, 2001).

Two kinds of transmission can occur in poultry:

1. **Vertically**, the transfer of a disease, condition, or trait from one generation to the next,
    a. SE can be transmitted by the upper and or lower reproductive tract of the infected hen (Cox et al., 2000; Gast et al.' 2013; Guard-Petter, 2001). When the upper or lower reproductive tract of the hen is contaminated, the shell of the egg is contaminated. When examining naturally contaminated hens, the incidence of SE in eggs is low and the number of infected chicks is low (Cox et al., 2000; Series, 2002). This means that an infected hen would contaminate a very small number of eggs and chicks through vertical transmission.
2. **Horizontally**, transmission of infection by contact.

Infections in poultry can come from infection in the parent stock or from contact with infected humans, animals, insects or a contaminated environment (Guard-petter, 2001).

The microbiological safety of poultry meat is dependent on a number of factors such as hygiene (including pest control) and infection levels on the grandparent farms, multiplier farms, hatcheries, broiler farms, feed suppliers, abattoirs and meat cutting and processing units, storage and distribution conditions and final handling of the poultry products by the consumer.

2.1.2. *Salmonella enteritidis* - A foodborne disease in humans

Most salmonella live in the intestinal tract of animals and birds and are transmitted to humans when faeces from animals, directly or indirectly, contaminates foods that humans eat (Figure 1) (CDC, 2013). SE is most common in animal food sources such as eggs, meat and dairy.

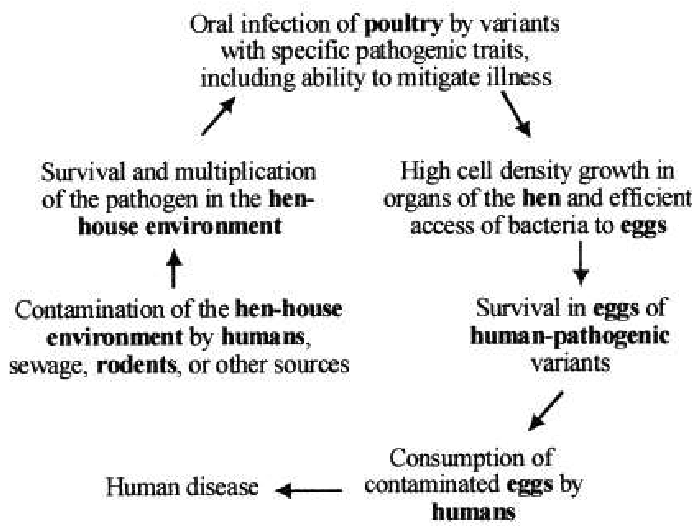

Figure 1 (Guard-petter, 2001) : The route to human infection by *Salmonella enteritidis*.

The dose required to cause illness in humans varies dependent on the person who consumes the contaminated product. Susceptibility is highest in young, older and immuno-compromised people (CCFH, 2007). The dose required ranges from 4-10^7 cells but could be more for non-typhoidal salmonella (CCFH, 2007).

In humans, clinical signs of diarrhoea begin 12 to 72 hours after consuming a contaminated product. The illness usually lasts 4 to 7 days, and most people recover without antibiotic treatment. The elderly, infants, and those with impaired immune systems may have a more severe illness which could result in septicaemia and death if the patient is not treated with appropriate antibiotics (CDC, 2013). The years of life lost as a result of HIV, and associated diseases (Tuberculosis and AIDS) is a leading cause of disease in South Africa (Groenewald, Bradshaw, Day & Laubscher, 2017). In 2015 diarrhoeal diseases were among the top ten leading causes of years of life lost per province for every single province in South Africa apart from the Western Cape(Groenewald *et al.*, 2017). In people with a compromised cell mediated immunity non-typhoidal salmonella (NTS) infection are severe, reoccurring, septicaemic and potentially deadly (Gordon, 2008; de Jong *et al.*, 2012).

One of the important risk factors to consider in treating salmonellosis is antimicrobial resistance (AMR) of SE as this presents an obstacle in treating salmonellosis in humans in cases where antibiotics are needed (Zishiri, Mkhize & Mukaratirwa, 2016).

Salmonella enteritidis is of global concern, therefore the level of detection and / or surveillance have been increasing globally. There were increasing isolates of SE from human cases of salmonellosis in Thailand from 1976 to 1993 (Sakai & Chalermchaikit, 1996), Argentina from 1987 to 1991 (Caffer & Eiguer, 1994), Italy from 1982 to 1992 (Fantasia & Filetici, 1994) and the Western Cape and Gauteng Provinces in South Africa in 2011 and 2012 (Keddy, 2013a).

The prevalence of SE varies dramatically in different countries. Salmonella was the most common, bacterial origin, confirmed aetiologic agent which caused disease of humans in the United states of America in 2007 and SE was the most prevalent serotype isolated in these cases (CDC, 2013). Hue et al. reported an isolation rate of 7.52% of salmonella on broiler carcasses originating from 14 abattoirs in France in 2008, 2.08% of the broiler carcasses sampled were detected SE positive (Hue et al., 2011). Ravel et al.(2010) in a study area from Canada reported that 7% of retail chicken breast samples tested positive for SE while 21.8% of human cases of salmonellosis were caused by SE (Ravel et al., 2010). They also reported seasonality associated with cases of SE, which may have been due to barbeques and gardening (Ravel et al., 2010). In the Lusaka district of Zambia, 100 swabs were taken from broiler carcasses at two abattoirs and tested for salmonella (Shamaila, Ndashe, Kasase, Mubanga, Moonga, Mwansa & Hang'ombe, 2018). Of the 100 samples, two were positive for Salmonella spp. In South Africa, a study conducted in 2014, of virulence and antimicrobial resistance genes in Salmonella spp. where 200 caecal samples were collected from chicken abattoirs in the Durban metropole, Kwazulu Natal Province found that 51% of the samples were positive for *Salmonella spp.* and 68.6% of the salmonella detected were *Salmonella enterica* (Zishiri *et al.*, 2016). Zirishiri showed that there was resistance to tetracycline, trimethoprim-sulfamthoxazole, trimethoprim, kanamycin, gentamicin, ampicillin, amoxicillin, chloramphenicol, erythromycin and streptomycin.

2.1.3. Pathway

The World Health Organisation describes the pathway of production to consumption of salmonella starting at the abattoir and ending at the consumer (Series, 2002). The Codex Alimentarius Committee on food hygiene working group established guidelines for the control of *Campylobacter* and *Salmonella spp*. in broiler chicken meat and specifically stated that more data is required from processing facilities used in different countries (CCFH, 2007).

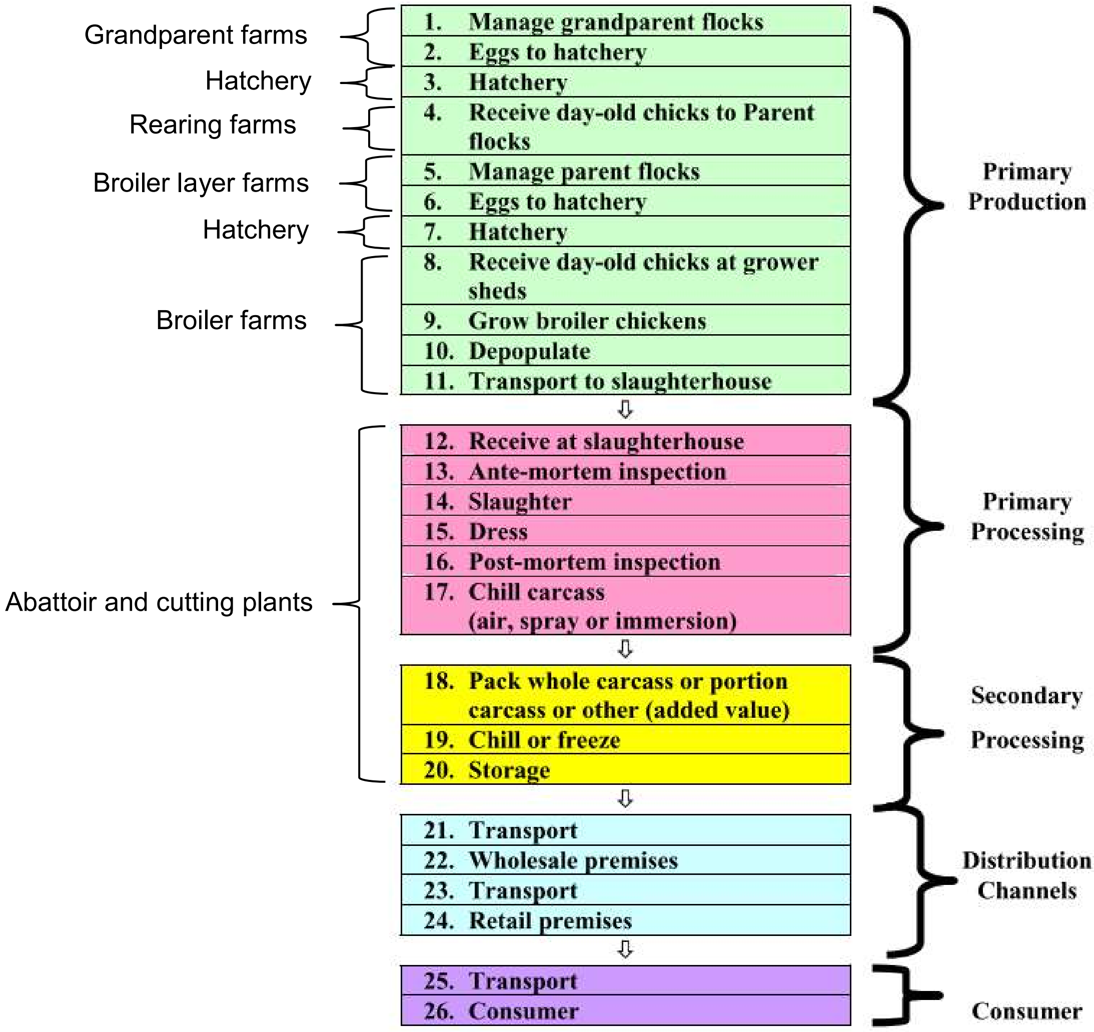

Figure 2 Farm to table continuum with labels for farm type (CCFH, 2007)

Figure 2 refers to the flow of poultry through the food chain with the main production steps in the chain. Poultry flows from breeder or multiplier farms to hatcheries where the eggs of the breeders or multipliers are hatched. The chicks from the breeders are taken from the hatcheries to rearing farms. Chickens that are at the point of lay are taken from rearing farms to broiler layer farms. The eggs are taken from broiler layer farms to hatcheries and the chicks from hatcheries are taken to the broiler farms where they are raised to slaughter size. Once the broilers are at slaughter size they are transported to the abattoir where they are slaughtered. From the abattoir, the carcasses can go directly to distribution or they can be taken to a cutting plant for further processing. Figure 5 has the main risk factors associated with SE contamination while Figure 2 has the characterisation of processes as described by the Codex Committee on Food Hygiene (CCFH) Working Group on Guidelines for control of *Campylobacter* and *Salmonella spp*. in broiler (young bird) chicken meat (CCFH, 2007).

2.1.4. Epidemiological Risk factors

Guard-Petter, listed epidemiological risk factors described by different authors that can cause an increase in the probability that an egg producing poultry farm could be producing SE contaminated eggs. Alterations in gut flora and a decrease in competitive exclusion are precipitated by management practices such as, moulting by withholding feed and water (Guard-Petter, 2001). The stocking of the hen house with chicks from farms with unknown SE status (Guard-Petter, 2001) has been shown to increase the probability of SE infection within the flock. The presence of other infectious agents of chickens may exacerbate problems and increase the probability to produce SE contaminated eggs (Deitch, 1987; Deitch et al., 1987; Phillips & Opitz, 1995). A poorly controlled rodent population increases risk as rodents can be carriers of SE or act as fomites for the spread of SE (Davies & Wray, 1995; Davies & Wray, 1996). Poor ventilation and dusty environments are risk factors especially at a young age as the bacteria enter the chick via the respiratory tract (Humphrey et al., 1992; Holt et al., 1998).

The association between poultry density and Salmonella infection in commercial laying flocks was described in Iran by Ansari et al. (2017). They showed that a high number of birds on a farm is an important risk factor for Salmonella contamination of eggs. They postulated that high number of birds per farm is not only a crucial factor at the farm level but also is important at the area level. In other words, when there are several farms with high poultry density near to each other the chance of salmonella contamination may increase (Ansari et al., 2017).

Cox et al.(2000) described additional causes of horizontal transition in poultry. Eggs laid in wet, dirty nests or on the floor are more likely to be contaminated with SE. Due to the fact SE is an inhabitant of the gut and upper and lower reproductive tract of the hen (Cox, Berrang, & Cason,

2000) this leads to bacterial inoculation as bacteria are capable of penetrating the of inner membranes and albumen of eggs on the first day of inoculation (Cox et al., 2000).

Van Hoorebeke et al. (2011) in a review article described various factors related to housing that correlate to SE introduction and detection on a farm (Van Hoorebeke, Van Immerseel, Haesebrouck, Ducatelle, & Dewulf, 2011). The presence of multiple flocks on one farm, especially when the different flocks and laying hen houses on the farm are linked through egg conveyor belts, feed pipes, passageways personnel and equipment as this leads to cross contamination and spread between houses (Carrique-Mas et al., 2008). Layer hens of different ages on the same farm and high number of hens in a flock in laying hens lead to an increase in probability of isolation of SE (Carrique-Mas, Breslin, Snow, Mclaren, Sayers & Davies, 2009; Carrique-Mas, Marín, Breslin, McLaren & Davies, 2009).

The immunosuppressive effect of stress has been shown in laying hens. This can have negative consequences with respect to Salmonella infection and shedding. Stressful periods that could cause an increase in shedding were identified as moving from the rearing site to the egg producing plant, the onset of lay, final stages of the production period, thermal extremes, transportation to the slaughter-house and induced moulting (Van Hoorebeke *et al.*, 2011).

A previously infected farm increases the probability of infection as SE can infect the insect population, pests, litter beetle (*Alphitobius diaperinus)* (Baggesen, Olsen & Bisgaard, 1992) as well as rodents and workers of the farm (Carrique-Mas, Breslin, *et al.*, 2009; Davies & Wray, 1995; Giessen, Ament & Notermans, 1994; Øystein, Skov, Chriél, Agger & Bisgaard, 1996). A previously infected farm will have a contaminated environment and equipment if the farm and equipment have not been cleaned with proven cleaning procedures and chemicals (Giessen *et al.*, 1994; Rose, Beaudeau, Drouin, Toux, Rose & Colin, 2000). Older farms are more likely to have been infected previously (Van Hoorebeke, Van Immerseel, Schulz, Hartung, Harisberger, Barco, Ricci, Theodoropoulos, Xylouri, De Vylder, Ducatelle, Haesebrouck, Pasmans, de Kruif & Dewulf, 2010). The presence of poultry red mite (*Dermanyssus gallinae*) and the presence of helminths also increases the risk of infection with SE (Kowalski & Sokół, 2009; Moro, Fravalo, Amelot, Chauve, Zenner & Salvat, 2007; Valiente Moro, De Luna, Tod, Guy, Sparagano & Zenner, 2009), due to the stress these parasites induce as well as their effect on blood protein and thus immunity.

The largest part of infection arising at hatcheries is as a result of horizontal transmission (Cox *et al.*, 2000) . The chicks hatching above and below a Salmonella infected chick were highly likely to become infected, If there was external contamination of the egg's shell or shell membrane contamination can lead to infection of chicks at pipping (When the chick starts to hatch) and the increased incubation temperatures allow the salmonella to multiply within the eggs

In review articles by Rose et al. (1999) and Le Bouquin et al. (2010) risk factors for SE in a broiler farm were given. In addition to some of the risk factors previously highlighted such as Salmonella contamination of the previous flock with persistence inside the house, the presence of rodents and Salmonella contamination of the house they mentioned, poor hygiene management (Fris & van den Bos, 1995; Henken, Frankena, Goelema, Graat & Noordhuizen, 1992), infected day-old chicks (Christensen, Brown, Madsen, Olsen & Bisgaard, 1997; Davies, Nicholas, McLaren, Corkish, Lanning & Wray, 1997; Øystein *et al.*, 1996; Rose *et al.*, 1999), feed contamination with SE (Christensen *et al.*, 1997; Davies *et al.*, 1997; Øystein *et al.*, 1996), more than 3 houses per farm (Øystein *et al.*, 1996), rainy cold season (Øystein *et al.*, 1996), presence of bell drinkers at day 1 (Rose et al., 1999), lack of control of infection status of day old chicks (Rose et al., 1999), parking of the feed truck near the change room (Rose et al., 1999), contamination of vehicles and foot wear (Rose et al., 1999, 2000) and non-heat treated feed vs heat treated feed on day one (Rose et al., 1999).

Hue et. al. (2011) identified the that the presence of more humans at the evisceration section of the slaughter line and adjustment of the evisceration machine according to the bird size to prevent contamination of the carcass with gut content decreased the probability of detecting SE at abattoirs. The following factors increase the probability of SE detection at abattoirs: mechanical (automatic) evisceration, disinfection of trucks between each round, sampling during the second half of slaughter and systematic external carcass rinsing (Hue *et al.*, 2011).

2.2. Mitigation

Various strategies have been used to control SE in the human population. These include legislative changes such as compulsory processing of infected meat, compulsory reporting of SE detection, and slaughter of infected grandparent flocks. Below are some examples of where interventions done to control SE in poultry and humans that had a positive effect.

Foley et al. (2008) reported that it was possible to control a specific serotype of salmonella with a holistic approach as there was a decrease in the number of isolates from human cases from the USA caused by SE over the period 1995 to 2004 (Foley, Lynne, & Nayak, 2008). The USA included SE in the egg and meat improvement plan in 1994 (Foley et al., 2008). As a result of the improvement plan there was an increase in the number of farms vaccinating for SE (Foley et al., 2008). Esaki (2013) reported that in Japan the detection rate of SE on egg layer farms decreased by half and that the foodborne diseases caused by Salmonella decreased from 1998 to 2010 by 90%. This was most likely due to making SE a notifiable disease, publishing an egg handling guideline and the introduction of an inactivated vaccine for SE (Esaki et al., 2013). Hue et al. (2011) also reported a decrease in prevalence from 1998 to 2010 and they postulated it was as a result of a legislative change in France in 1998 (Hue et al., 2011).

In the risk assessment of salmonella published by the World Health Organisation in 2002 it states that when management strategies that affect the level of SE contamination on chickens are implemented, the relationship to risk of illness in humans is estimated to be greater than a one-to-one relationship (Series, 2002). Therefore, it is important that the entire poultry production chain be part of a qualitative risk assessment.

Esaki et al. (2013) reported that the decrease in SE detected in Japan could have been because of *S. enteritidis* and *S. typhimurium* actively notified to the local authority (Esaki et al., 2013), the use of a standard for the handing of eggs based on hygienic practices (Esaki et al., 2013) and the administration of an inactivated Salmonella vaccine for *S. enteritidis* or *S. typhimuriumm* to broiler layers (Esaki et al., 2013), (Van Hoorebeke et al., 2011).

Cox et al. described that chemical disinfection needed additional research and that if chemical are applied to the hatching eggs it should be done as soon after lay as possible (Cox et al., 2000). They also listed a number of egg factors that have an effect on SE penetration and proliferation in eggs. The egg factors listed were: shell quality(Sauter & Petersen, 1974), pH (Sauter, Petersen, Parkinson & Steele, 1979), number of pores on an egg shell (Walden, Allen & Trussell, 1956), temperature (Graves & MacLaury, 1962), humidity and vapour pressure (Graves & MacLaury, 1962)

Factors at hatchery that can improve the control of SE are the use of clean disinfected eggs and supplier quality/disease assurance (Cox et al., 2000). Broiler farms could control SE infection are; control over day old chick salmonella status (Rose et al., 1999), terminal disinfection of the house with formaldehyde or glutar aldehyde containing disinfectant (Rose et al., 2000), the use of an external contractor to disinfect the house (Rose et al., 2000), the use of approve disinfectants (Rose et al., 2000) and the use of antibiotics when chicks could be infected (Le Bouquin *et al.*, 2010; Heyndrickx, Vandekerchove, Herman, Rollier, Grijspeerdt & De Zutter, 2002; Hue *et al.*, 2011)

Decreased detection of SE at abattoirs are more common (Hue *et al.*, 2011) when abattoirs slaughter other species, collect samples during the first half of slaughter, external carcass rinsing is done only if needed or no rinsing at all, the slaughter of free range and label chickens and the attribution of a reference number to the flock at arrival.

2.3. **Risk analysis**

In addition to its food borne disease status, SE is an organism causing a trade sensitive disease according to the World Animal Health Organisation (World Organisation for Animal Health, OIE). This results in trade restrictions in accordance with the Sanitary and phytosanitary agreement of the World Trade Organisation (WTO).

The World Trade Organisation, WTO is an international organisation made up of countries that have signed agreements regarding international trade. South Africa is part of the WTO and as such has agreed to the standards of the organisation. South Africa should therefore perform risk analyses based on the Sanitary and Phytosanitary Agreement (SPS Agreement, 1995) on local broiler meat to determine what the risk of SE is. A risk assessment, performed in this manner, will aid decisions on the acceptable level of risk for trading partners.

For the standards, guidelines and recommendations the relevant international guiding standards should be referred to. According to the SPS Agreement, these are the Codex Alimentarius Commission, the OIE code and the IPPC (International Plant Protection Convention).

2.3.1. The Codex approach to risk assessment in food safety

"The Codex Alimentarius or "Food Code" is a collection of standards, guidelines and codes of practice adopted by the Codex Alimentarius Commission (CAC) (Codex Alimentarius Commission, 2014). The Commission is the central part of the Joint Food and Agriculture Organisation, FAO and World Health Organisation, WHO Food Standards Programme and was established by FAO and WHO to protect consumer health and promote fair practices in food."(Codex Alimentarius Commission, 2014) As poultry products are part of the food chain, reference to the CAC approach to risk assessment is highly relevant.

The assessment consists of four steps and a number of principles (Codex Alimentarius Commission, 2001): (1) Hazard identification, to identify the pathogen that is causing the disease; (2) Hazard characterization, to evaluate the nature of the pathogen, and estimate the dose response; (3) Exposure assessment, to describe the biological pathway(s) necessary for the exposure of animals and humans, and to estimate the likelihood of this exposure occurring; (4) Risk characterization, which consists of integrating the results from the hazard characterization and the exposure assessment to develop an estimate of the probability of occurrence and severity of the disease in a given population.

2.3.1.1. Risk analysis and the use of Hazard Analysis and Critical Control Points (HACCP)

Referring to the general principles of food hygiene published by the Codex Alimentarius Commission in 2003 (Codex Alimentarius Commission, 2003). HACCP is defined as a system that identifies, evaluates, and controls hazards, which are significant for food safety. It is a process control tool (Notermans et al., 1996). There are seven principles of HACCP defined as follows:

1. Conduct a hazard analysis.
2. Determine the Critical Control Points (CCPs).

3. Establish critical limit(s).
4. Establish a system to monitor control of the CCP.
5. Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
6. Establish procedures for verification to confirm that the HACCP system is working effectively
7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

A hazard is defined as a biological, chemical or physical agent in, or condition of, food, with the potential to cause an adverse health effect (Codex Alimentarius Commission, 2003). Hazard analysis is defined as the process of collecting and evaluating information on hazards and conditions leading to their presence, to decide which are significant for food safety and therefore should be addressed in the HACCP plan. The significance and the extent of control are dependent on the intended use of the product which is based on the end user or consumer. The HACCP plan is defined as a document prepared in accordance with the principles of HACCP to ensure control of hazards, which are significant for food safety in the segment of the food chain under consideration. A critical control point is defined as a step in a process, that is essential to prevent or eliminate a food safety hazard, at which a measurable degree of control can be applied and or achieve a quantifiable reduction to an acceptable level and therefore a safe food product on a quantitative basis is produced (Notermans & Meadb, 1996). The document goes on to define how a HACCP system should be implemented and critical limits should be set for critical control points (Principle 3), critical limits should be monitored (Principle 4). Therefore a critical control point is a step in a process that materials pass through where control is applied, measured and recorded and corrective actions are taken when the measurements are out of specific critical limits.

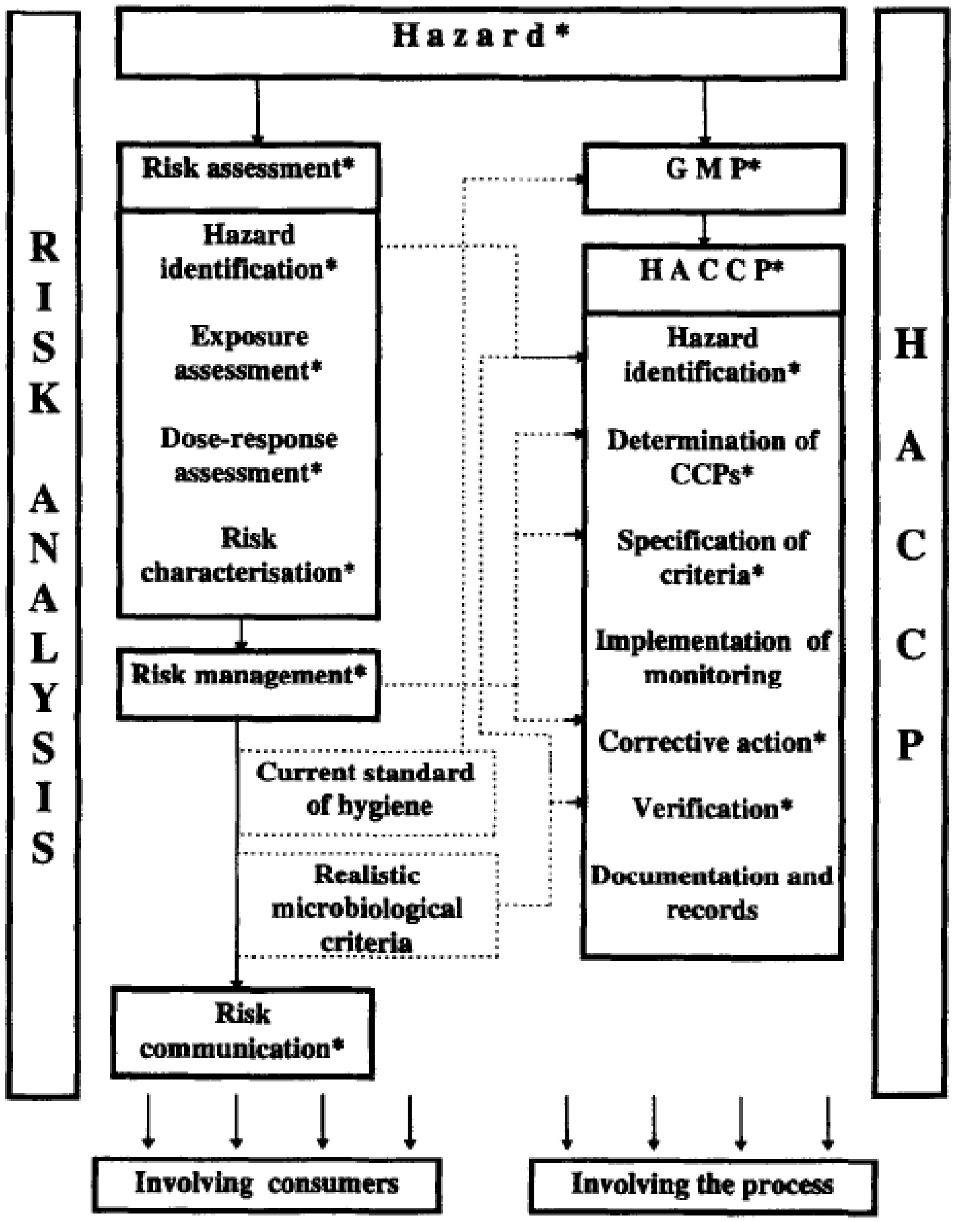

Figure 3 Interactions between risk analysis and HACCP (Notermans *et al.*, 1996)

Notermans et al., 1996 put forward a glossary of terms about risk analysis and food safety systems that can be used with food products and consumer protection. These terms are a suitable standard that will be understood by food producers. As HACCP is a process control tool it is part of risk analysis (risk management refer to figure 4 which shows that most of the components of HACCP are part of risk management).

In HACCP the use of qualitative risk analysis (Principle 1 of HACCP) is applied to determine steps in a process where a quantifiable reduction in a specific hazard occurs. As in risk analysis, the process is broken down into discrete steps. As producers often only have control of the part of the food chain under their direct control, the risk analysis conducted in HACCP is focussed primarily on the raw material received based on predefined raw material supplier specifications and the processes at the facility applying the HACCP system. The HACCP system takes into account any risk that the final product is likely to pose to the consumer of that product. Risk analysis (as defined by CAC) is, however, for the entire food chain and for a specific defined hazard according to the scope of the risk analysis.

Notermans et al.(1996) introduces the notion of steps in a process where the measurement of risk reduction is not always possible or practical. The example of evisceration of a chicken carcass was used where contamination of the carcass with intestinal content would result in an increase in Salmonella load and that this could be mitigated to some extent by correct operation of the equipment (Notermans & Meadb, 1996). The effect of correct operation would be difficult to quantify and the step of evisceration will never reduce the salmonella hazard to acceptably safe levels. In HACCP, the use of critical control points, it is not possible at such a step. Therefore, in HACCP there is a need to have programs in place to ensure the correct operation of equipment. The risks present in such a step are to be mitigated elsewhere in the food chain either at the farms before the chicken reaches the abattoir or at the food-processing establishment where the chicken is cooked. An example of controlling salmonella in raw poultry products would be to slaughter chickens that come from farms free of salmonella.

In the case of ISO22000 the facility would need to provide sufficient information to the consumer so that the consumer is equipped to mitigate the risk that the product could contain.

The basic operations that create a hygienic environment are known as prerequisite programs (PRP). These are things like maintenance, cleaning, worker health, correct operation of equipment and supplier quality assurance. These basic preventative measures must be in place before HACCP can be implemented. These operations are hard to quantify but have an effect on the final product safety.

HACCP is a process control method that has an element of risk analysis with a focus on the reduction of the probability that a final product could contain any thing hazardous to human or animal health. Risk analysis in terms of Codex is a systematic evaluation of the food chain to ascertain the probability that a specific product could cause a health problem because of a specific hazard.

The article published by Notermans et al. in 1996 does not account for the newer process control method approaches. An example of this is the ISO 22000 standard. This allows food producers to have multiple controls, not necessarily critical controls, whose summation of control leads to the production of an acceptable product. ISO 22000 accounts for the intended use of the product for the next step in the food chain, and not necessarily the end user, when the amount of acceptable risk is to be determined. In this standard, emphasis is put on communication of risks, that product is expected to contain, to the consumer of the product. Communication of the risk across the food chain is also expected by ISO 22000. In other words, the person or customer receiving the raw material or product is to know exactly what risks are expected to be in the material and what can be done to mitigate the risks. Labelling of the product is the normal communication method used.

2.3.2. The OIE approach to risk assessment

The OIE Terrestrial Animal Code encompasses the international regulations with regard to the application of the SPS Agreement concerning risk analysis as applied to importation of animals and animal products (OIE, 2010).The SPS Agreement sets out the level of protection applied to imported products and that these should not be stricter than the level of protection applied to local products, also that the risk of an imported product to that of a local product should be comparable (OIE, 2010).

The handbook describes the questions that should be answered during a risk analysis.

1. What can go wrong?
2. How likely is it to go wrong?
3. What are the consequences of it going wrong?
4. What can be done to reduce the likelihood and/or the consequences of its going wrong?

The risk analysis approaches of OIE and Codex Alimentarius were selected and are compared in table form (Table 2). Both approaches have the same components but vary in the order of application and in their definition.

Table 2: A comparison of risk analysis using Codex and OIE

Approach							
Codex Alimentarius Commission (Codex)				World organisation for Animal Health (OIE , 2010; Codex Alimentarius Commission, 2007)			
Risk analysis	Risk Assessment	Risk communication	Hazard Identification	Risk analysis		Risk communication	Hazard identification
			Hazard characterisation		Risk Assessment		
			Exposure assessment				Entry Assessment
							Exposure assessment
			Risk characterisation				Consequence assessment
			Risk estimate				Risk estimation
	Risk management				Risk management		Risk evaluation
			Option assessment				Option evaluation
							Implementation
			Monitoring and review				Monitoring and review

As the two approaches are very different, a comparison of the main terms used is presented below.

- **Hazard Identification**

Codex use of hazard identification simply involves the identification of hazards whereas the OIE definition includes a step for the categorisation of the hazard into potential hazards or not.

- **Hazard Characterization**

Codex: “The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food” (Codex Alimentarius Commission, 2016).

There is no term use in the OIE like this but it is closely linked to the Consequence assessment of the OIE risk analysis. The Codex definition excludes environmental consequences.

- **Consequence assessment**

OIE: “Consequence assessment consists of describing the relationship between specified exposures to a biological agent and the consequences of those exposures. A causal process should exist by which exposures produce adverse health or environmental consequences, which may in turn lead to socio-economic consequences. The consequence assessment describes the potential consequences of a given exposure and estimates the probability of them occurring. This estimate may be either qualitative (in words) or quantitative (a numerical estimate).”(OIE, 2013)

- **Exposure Assessment**

Codex: “The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant” (Codex Alimentarius Commission, 2016).

OIE: “Exposure assessment consists of describing the biological pathway(s) necessary for exposure of animals and humans in the importing country to the hazards (in this case the pathogenic agents) from a given risk source, and estimating the probability of the exposure(s) occurring, either qualitatively (in words) or quantitatively (as a numerical estimate).”

The Codex definition is linked to two OIE definitions of Entry assessment and Exposure assessment but it does not deal with effects in terms of environmental impact and impact on animal populations.

- **Risk Estimate**

Codex: Output of Risk Characterization(Codex Alimentarius Commission, 1999). “Contains a description of uncertainty and where the uncertainty arose during the Risk Assessment process.”

OIE: “Risk estimation consists of integrating the results from the entry assessment, exposure assessment, and consequence assessment to produce overall measures of risks associated with the hazards identified at the outset. Thus risk estimation takes into account the whole of the risk pathway from hazard identified to unwanted outcome” (OIE, 2016)

The OIE risk estimation definition is a combination of the Codex risk characterisation and risk estimation.

Specific definitions according to the OIE:

- **Entry assessment**

There is no similar term in the Codex as it does not have the same focus.

In this study the approach and definitions would be the same as that of the Codex as SE is a food safety risk.

2.4. Tools for assessing risk

According to Mossel et al. (1998) it is not possible to reach an acceptable level of risk with only final product testing when the risk is a bacteria or a bacterial derived toxin (Mossel, Weenk, Morris, & Struijk, 1998). The bacteria go into a state of arrest, a sub lethal state, when they are stressed. The bacteria are not cultured but nor are they dead. If the bacteria are given an environment conducive to growth, they will be able to grow again and cause disease. For the control of bacterial food safety hazards a longitudinally integrated forward intervention strategy, validated by final product testing during its elaboration and implementation, should be required (Mossel et al., 1998).

In the elaboration of microbiological target or reference values for foods, much debate has centred on the use of the terms ‘‘zero’’ or ‘‘nil’’ in relation to tolerance (Farber et al., 1996; Hitchins, 1996). The practical meaning of this designation is nonetheless clear. It conveys that if n samples of x grams of a food are examined for the target organism by prescribed methodology, that organism will not be isolated. Depending on the vulnerability of the consumers assumed to ingest the product (Mossel et al., 1998) the target level can be adjusted. It may be worthwhile to reassess the level of acceptability in cases where immunity is compromised. Given the level of susceptibility of population in South Africa this adjustment may be needed.

Chapter 3: **Materials and methods**

3.1. **Study design**

The risk assessment was developed using the methodology described by the CAC and an adaptation of the method from L. De Sadeleer et al., (2009 (Sadeleer & Dewulf, 2009) but excluding the consumer.

This study used a similar pathway as that described by Schlosser et. al. (2003) where the flocks are included in the pathway (Schlosser et al., 2003). The main difference was that the multiplier flocks (Broiler layer flocks) were included as egg producing flocks, in this study, and considered to have similar risk factors as table egg laying flock.

In South Africa all, the abattoirs are legally obliged to register and be approved by the local veterinary authority. The number and location of all poultry abattoirs is known. The number of farms and hatcheries in the Western Cape was not known at the start of the study and this information was requested from the abattoirs as part of the survey.

In order to get information about the food chain, the risks and control measures for SE, a questionnaire was developed for every step in the production chain namely: abattoirs, broiler farms, hatcheries, broiler laying farms and broiler layer rearing farms. The possible influence of the production steps on *Salmonella* contamination were identified using literature review for different known risk factors and control measures.

Historical data for 6 years was obtained from the WCDoA. This data contained the location and the number of samples that were positive for SE.

3.2. **Questionnaire**

The questionnaire was based on work previously published (Ansari *et al.*, 2017; Baggesen *et al.*, 1992; Le Bouquin *et al.*, 2010; Carrique-Mas, Breslin, *et al.*, 2009; Carrique-Mas, Marín, *et al.*, 2009; Christensen *et al.*, 1997; Cox *et al.*, 2000; Davies & Wray, 1995, 1996; Deitch, 1987; Esaki *et al.*, 2013; Foley & Lynne, 2008; Foley *et al.*, 2008; Giessen *et al.*, 1994; Graves & MacLaury, 1962; Guard-Petter, 2001; Holt *et al.*, 1998; Van Hoorebeke *et al.*, 2011; Humphrey *et al.*, 1992; Kowalski & Sokół, 2009; Øystein *et al.*, 1996; Phillips & Opitz, 1995; Rose *et al.*, 1999, 2000; Series, 2002; Valiente Moro *et al.*, 2009) and covered in the literature review of risk factors for the introduction and replication of SE. The questionnaire was reviewed by one poultry veterinarian (and one state veterinarian involved in the control of poultry diseases. The poultry veterinarian (Dr Marie Oosthuisen) is actively involved in the poultry industry. The state veterinarian (Dr Sewellyn Davey) has been involved in the control of SE and other poultry diseases in the state for over 10 years. There were insufficient abattoirs to use some of them to test the questionnaire and therefore being obliged to remove them from the study. Comments about the suitability and understand-ability were requested from the veterinarians for further refining of the questionnaire.

For some of the larger companies it was not possible to make use of the online questionnaire and an additional Microsoft Excel spreadsheet questionnaire was developed for each part of the production process. The spreadsheet questionnaire had identical questions without the controls, such as dropdowns and relevance equations. The larger companies choose to either use the online version or the spreadsheet version.

Open discussions were had during the administration of the questionnaire with the person responsible for the abattoir, farm of farms. This was to get additional data and confirmed interpretation of responses.

3.2.1. Questionnaire design

A conceptual model of the commodity chain along with the factors that impact on each step of the chain was developed to assist in planning for the questionnaire.

All processes in the poultry production chain were contained in the same electronic questionnaire. The questionnaire was developed using LimeSurvey: An Open Source survey tool. The data was hosted on the WCDoA server for security reasons. The questionnaire covered topics such as type of operation, infrastructure, hygiene, contact with birds and movements of birds, flock health and management practices.

Relevance equations were used to show or hide specific questions based on the responses of the person completing the questionnaire.

The questionnaire was divided into the following sections:

- Informed consent - The respondent gives consent to participating in the survey,
- General characteristics and structure - The type of operation or farm, location, size and general description,
- Farms with multiple houses or sites - Connections between houses number as well as size of houses
- Management practice questions for farms - Vaccinations, cleaning, pest control and feeding questions
- Hatchery specific questions- Questions specific for hatcheries
- Layer management questions - Questions specific to layer farms and the management of layers
- Supplier quality assurance - Questions about the suppliers of chickens or eggs as well as feed suppliers
- Sampling - General bacteriology sampling questions

- Cleaning program - Frequency, methods, and cleaning chemicals used for cleaning are requested
- General hygiene and Biosecurity - Questions about training, hygiene management systems, and level of access to other poultry
- Visitors and contractors - Questions about the number and type of people visiting the site as well as the level of access to the chickens
- Abattoir and cutting plant specific questions - Abattoir specific questions related to processing of live chickens into meat, welfare, slaughter technique, meat inspection and chilling questions are asked.

3.2.2. Questionnaire administration

All questionnaire data were collected between September 2017 and September 2018.

3.2.2.1. Primary and secondary processing

There are 21 active registered poultry abattoirs in the Western Cape that are together registered for slaughtering 679650 chickens per day (Leask, 2017). Nine abattoirs registered to slaughter less than 250 poultry units per day each and therefore were excluded from the study. The 12 remaining abattoirs, together registered for slaughtering 679050 chickens per day, and registered for more than 400 poultry units per day each represent the majority of poultry meat produced in the Western Cape, excluding informal slaughter.

The resources available for the study were not adequate to sample at all abattoirs. A convenience sample of five abattoirs was selected, approved for 369850 poultry units per day.

At the abattoirs, the questionnaire was administered by the study leader with the hygiene or abattoir manager. In each abattoir, data related to the product flow and the process was obtained by administering the questionnaire.

3.2.2.2. Primary production

The head office of the veterinary or farmer health sections of the larger production groups completed the data for broiler farms under their control. For private farmers the questionnaire was administered over the phone by the study leader with the farmer.

For the hatcheries, broiler layers, rearing farms and grandparent farms the data came from the company head office who compiled a response for all of the farms under their control. There were no independent broiler layers, rearing farms, hatcheries or grandparent farms. All of these levels were controlled by larger production groups.

3.3. **Sampling and Testing**

Neck skin as it is a small inexpensive sample that represents the most contaminated portion of the carcass was used.

Samples were collected from the abattoirs for at least 40 farms that were included in the questionnaire.

This sample size will allow to determine the farm prevalence based on the more constraining expected prevalence of 50% with an absolute precision of 7.3% for a confidence of 95% using the following formula (Dohoo, Martin & Stryhn, 2010).

$n=Za^2*P*(1-P)/Pa^2$

With Za the value of Z in the normal distribution (with the accepted error being 5%, confidence 95% a=2.5%, Za= -1.96), P the excepted prevalence and Pa the absolute precision.

At least 10 individual samples would be collect by the abattoir for each farm and in total it was expected that 400 samples would be collected.

After a short training, done by the study leader, on the method of aseptic collection the abattoir's hygiene manager or quality controller was requested to collect 10 separate neck skin samples of the birds in a delivery from each farm that supplies the abattoir. Samples were frozen in the -12 to -18 °C freezers pending collection from the abattoir. The neck skin samples were to be taken at the end of the production line after final chiller during the entire production day. For each sample, the abattoir was asked to identify the farm that the chickens originated from. For each sample either the producers company or the farm of origin was contacted to obtain the hatchery, broiler layer and broiler layer rearing farm information. This information was recorded along with the outcome of the sample testing.

The Western Cape Provincial Veterinary Laboratory cultured the samples and presumptive isolated salmonella. Presumptive Salmonella isolates were sent for biomedical confirmation and serotyping at the Onderstepoort Veterinary Institute.

Poultry meat preparations, poultry meat products and handling of the poultry products by the consumer was not investigated.

3.3.1. Bacteriological laboratory analysis

The Western Cape Provincial Veterinary Laboratory (WCPVL) in Stellenbosch provided information about the culture methods used as it forms part of their laboratory manuals.

The detection of *Salmonella* necessitates four successive stages pre-enrichment in non- selective liquid medium, enrichment in selective liquid media, plating out and identification

Pre-enrichment is often necessary to permit detection of injured Salmonella. It is done by incubating the sample in buffered peptone water (also used as diluent) at 37 °C ± 1°C for 18h ± 2h.

Salmonella may be present in small numbers and are often accompanied by considerably larger numbers of other members of Enterobacteriaceae, therefore selective enrichment is necessary. Rappaport-Vassiliadis medium with soya (RVS broth) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn broth) selective media are inoculated with culture obtained from pre-enrichment. The broths are incubated at 41.5°C ± 1°C for 24 h ± 3 h and 37°C ± 1°C for 24 h ± 3 h respectively

Two selective solid media (Modified brilliant green agar (BGA) and Xylose Lysine Deoxycolate agar (XLD medium)) are plated with the cultures obtained above and are inoculated at 37°C ± 1°C for 24h ±3h.

Characteristic colonies, considered as presumptive *Salmonella*. The presumptive isolates were sent to the Onderstepoort Veterinary Institute in Pretoria, South Africa where the isolates were conformed to be salmonella using biochemical tests. The biochemical tests comprised reactions on triple sugar iron agar slants, Urea slants, broths of Dulcitol, Malonate, Lysine decarboxylase, lysine decarboxylase control and Thiogel. Thereafter serotyping of Salmonella based on the Kauffmann–White classification scheme, using slide agglutination principle involving different antisera to Salmonella was performed on the positive samples.

3.4. **Data analysis**

The results of the survey were compiled in MS excel.

Descriptive statistics were done using MS Excel 2016. This was done to describe the pathway of the disease and to characterise the processes in the food chain. All other statistical analysis were done using the software R (Copyright © 2004-2016)

Suppliers of eggs and chicks were requested and given in questionnaire responses which were used to do Social network analysis (SNA) in R. Interconnectedness of the various farms, abattoirs and hatcheries was calculated as part of the SNA.

As this is an assessment of SE in the poultry meat industry comparing industry practices to prevent SE with those in literature, the results of the questions were assessed individually. For those results where the question responses differed from each other and the variable is likely to be linked to the outcome of the bacteriology result (potential risk factor) a univariate odds ratio was calculated using the Miettinen method.

CI= OR^(1± 1,96/(Chisq^0.5)).

For the variables where the chi squared value was below 0.2 these variables were used in the principal component analysis.

Principle components analysis (PCA) is part of multivariate data analysis (Abdi & Williams, 2010; Wold, Esbensen & Geladi, 1987). PCA investigates a data table of variables that are interconnected with each other and helps to extract important information from the data table. It does this by creating a set of new orthogonal (thus independent) variables called principal components (Dimensions (Dim)) . PCA displays a pattern of similarity in the form of maps (Abdi & Williams, 2010). PCA maps allow viewing of variables in groups. Several sets of variables are therefore simultaneously studied. Taking into account the structure of the data will allow to balance the influence of each group of variables, study the links between the sets of variables and gives a graphical representation of the relations between (groups of) variables and between variables and individuals (farms or abattoirs). PCA was done using FactoMineR a package of R.

The map that represents the most variance in the analysis does not necessarily represent the positive salmonella farms the best therefore an additional map is created to best represent farm salmonella positivity. Therefore we used the 2 axis where the correlation with "Salm. positivity" was the highest (Dim2 and 4) to draw a map where we looked at the proximity or the distance with other factors that indicated some association(positive and negative respectively). These factors were selected for logistic regression. In order to validate the significance of the potential risk factor we used a general linear model with data family specified as binomial (Logistic regression). We used as dependent variable the results of the culture of the samples (positive or negative), the independent variables were the main risk factors selected from the PCA. AIC was used to select the simplest model in a stepwise backward procedure, until the smallest possible AIC (Akaike Index Criteria) was obtained. If the AIC increase when removing the factor then the factor was returned to the model. The objective was to have as few factors as possible in the model with the lowest possible AIC.

The Odds ratio was calculated from the general linear model (glm). OR=exp(coef) and Confidence interval = exp(coef+/- 1.96 std error)

As the data was binary data a Hosmer and Lemeshow goodness of fit (GOF) test was performed to see how well the model fits to the data collected.

A historical data set was provided by WCDoA Veterinary Services. The data contained all the reported cases of SE in the Western Cape from March 2012 to June 2018 and included the GPS location, the laboratory that did the testing and the date of diagnosis. The data set was cleaned and the identification of farms used in the sampling and survey data was applied.

Historical data was analysed to look for some spatio-temporal clustering. Ripley's K index analysis was conducted on the historical data to quantify non-random clustering patterns in space and time The analysis was conducted using the “splancs” package (Rowlingson & Diggle, 1996) in R. This Monte Carlo simulation was applied to the data to test for significance in the space time clustering. A Monte Carlo simulation function, uses a sum of residuals as a test statistic, randomly permutes the times of the set of points and recomputes the test statistic for a number of simulations to see if the outcome of the model is significant (Diggle, Chetwynd, Haggkvist & Morris, 1995).

Chapter 4: **Results**

One of the aims of this study was to conduct a risk analysis (RA) based on the Codex Alimentarius Commission standard for risk analysis. Therefore, it was agreed with supervisors to organise the results section in the shape of a RA report with some parts that were not completed during the study but still visible in the structure of the results.

4.1. **Hazard Identification**

Salmonella enterica subspecies enterica serotype Enteritidis (*Salmonella* Enteritidis, SE) was the target organism of this CAC risk analysis.

All the serotypes identified from the bacteriology were from the non-typhoidal serovars group.

4.2. **Hazard Characterization**

See *Salmonella enteritidis* - A foodborne disease in humans page 7 (Risk characterization are in the discussion page 51). No additional experiments or analysis on the hazard characterisation were part of this study.

4.3. **Exposure Assessment**

SE enters the human food chain at an abattoir when contaminated chickens are presented and SE not removed from the chicken during the slaughter process or the chicken carcass is contaminated by improper evisceration or cross contamination from another carcass, piece of equipment, carrier staff member, water, floor or packaging that has been previously contaminated.

Should the contaminated carcase be released for sale from the abattoir or cutting plant growth of the organism is enhanced by improper temperature control and unsafe handling by the consumer.

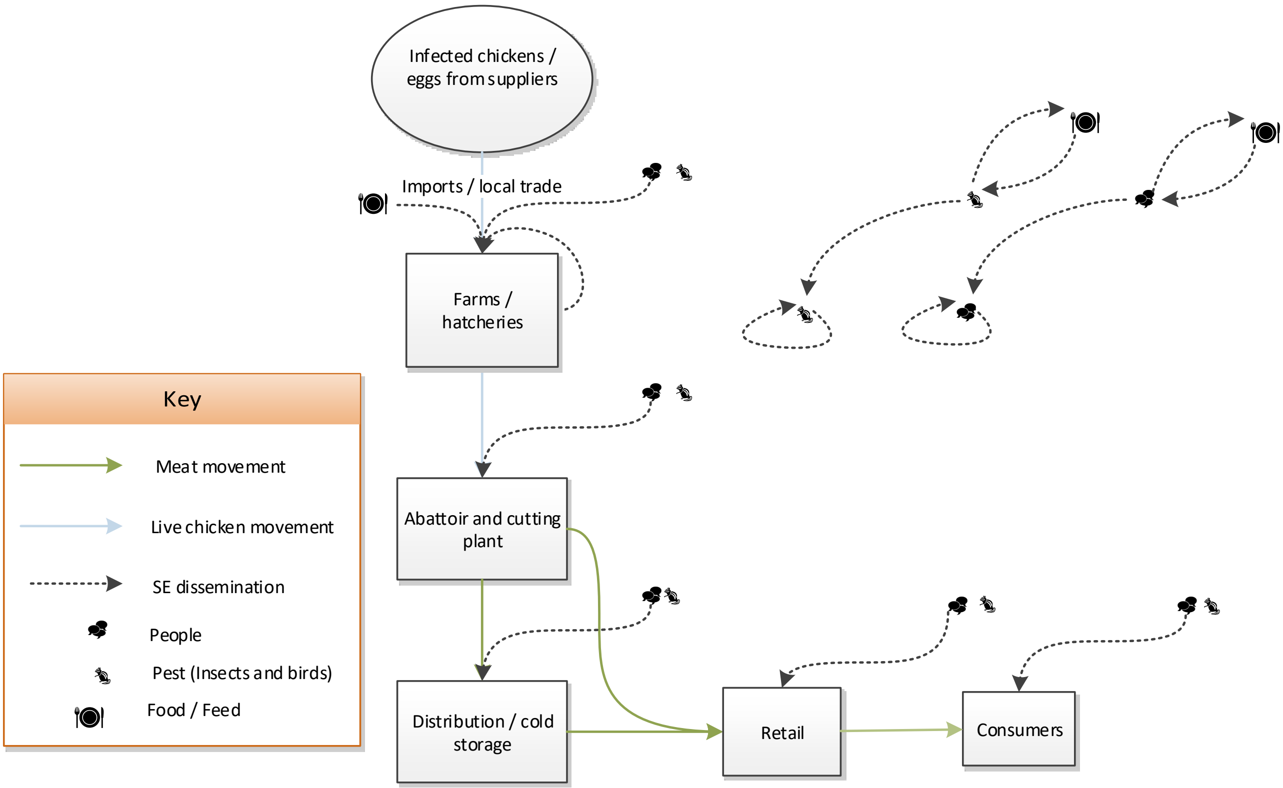

Figure 4 Concept model of the entry and movement of SE into the food chain

4.4. Risk Characterisation (1): Questionnaire design and results

A conceptual model wad derived from the literature review. The conceptualization was what assisted in customisation for each state of the production chain. This was used to design the questionnaire presented in annex C.

Questionnaire responses were received for 96 locations in the Western Cape broiler meat chain, which makes it possible to draw up an image of what broiler meat production is like in the Western Cape. Some of the locations had more than one part of the broiler chain contained at the same location.

During the course of the study, there was an outbreak of Highly Pathogenic Avian Influenza. This triggered the WCDoA to do a census of the poultry farms within the Western Cape (Roberts, 2018) and as a result, the location and sizes of all the larger broiler farms became available.

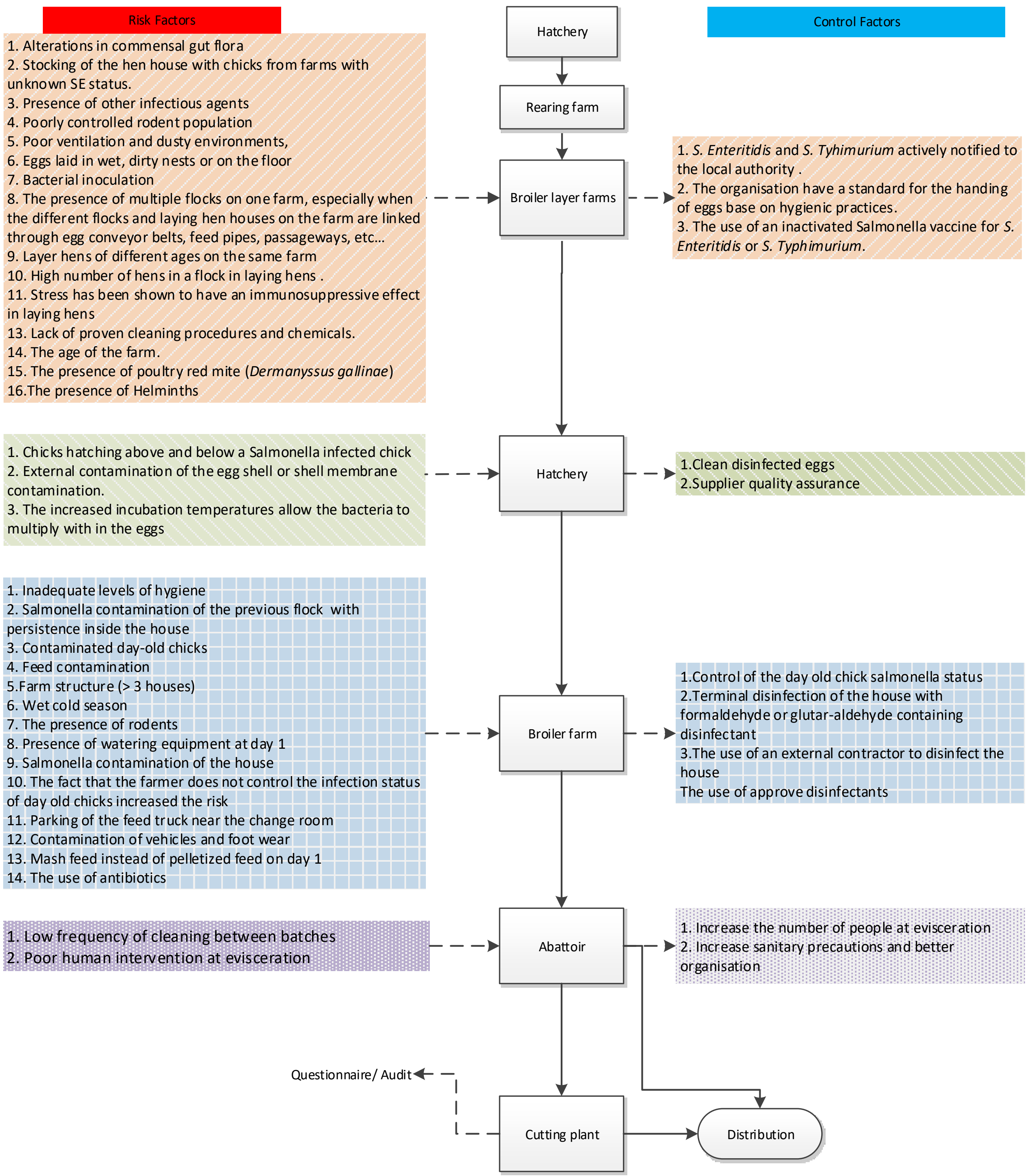

Figure 5 Poultry flow through the food chain

4.4.1. Characteristics and structure

Table 3: Western Cape Broiler Farm (Roberts, 2018) **and abattoir data** (Leask, 2017) **compared to survey respondents**

	Rearing farms	**Broiler layers**	**Hatcheries**	**Broiler farms**	**Abattoirs**	**Poultry units slaughtered per day**
Survey participants	13	20	7	53	5	369850
Western Cape	12	34	7	76	12	679050
Proportion participated	108.33	58.82	100	69.74	41.67	54.47

Table 3 shows the number of survey responses (n=96) compared to the total for the Western Cape. Poultry units per day is the sum of the poultry units that the abattoirs are legally permitted to slaughter in one day. A limitation of the farm data provided by the WCDoA Veterinary Services was that it did not permit a farmer to select multiple processes in the broiler chain. The farm was characterised by one process only. In the questionnaire, farmers were allowed to select more than one process if applicable. This is why the total responses (n=96) is less than the sum of all the responses for each process (n=98) and the number of rearing farms is more than the WCDoA number. There was only one response from a grandparent farm in the survey, which was not included for privacy reasons.

All the rearing, broiler layer farms and hatcheries that participated in the study formed part of larger companies or were contracted to grow or produce chickens or eggs for a larger company.

All of these farms had pest control programs, used SABS approved cleaning chemicals and made use of soft wood shavings for bedding received feed from feed factories with external certification for GMPs and culled smaller birds with cervical dislocation.

4.4.1.1. Rearing farms

There were 13 responses for rearing farms. Two (15.38%) of the farms also responded as broiler layer farms. One farm had an external audit for a documented management system (3rd party systems certification) and 83.33 % of the newest houses on the farms were older than 20 years.

Table 4 Rearing farm parameters

Factor	**Responded**	**Min**	**Average**	**Max**	**Std. dev**
Houses	13	2	5.92	16	3.8

Factor	Responded	Min	Average	Max	Std. dev
Total surface area in m^2 of all houses	13	2400	7046.92	19200	5193
Stocking density birds per m^2at full capacity	12	3.13	6.59	10.36	2.11
Birds are on the farm when the farm is in full production	12	8700	45919.33	195000	49034
Personnel with full time employment	12	4	8.5	21	5.04
Casual personnel	12	0	0.83	6	1.75

All of the rearing farms had multiple flocks on the farm. None of the farms reported being ever previously infected with *Salmonella*.

All of the farms fed a heat-treated feed on day one. The feed supply to all the farms was from feed companies with certified good manufacturing practices in place and all the farms collected feed samples for Salmonella testing. 8 of them gave the name (2 feed suppliers) the other 5 would have been another feed company.(3 different feed companies in total)

Water supplied to the farms came from underground or surface water. All the farms treated and tested the water for bacteriological safety.

The origin of the chicks raised at the rearing farms was provided for each farm. The companies supplying chicks were not part of the study due to their location and study resource constraints. Each farm only received chicks from one specific supplier. All of the farms requested the *Salmonella* status of the birds they receive. 92% of the farms sample the birds when they arrive and test for salmonella. 92% of the farms place chicks from multiple grandparent flocks into one house. All of the farms operate house on an all-in, all-out basis.

Cleaning and disinfection was done by operational staff (7.69%), external contractors (30.77) and separate teams of staff dedicated to cleaning (53.85%). Farms made use of contact plates and swabs for verification of cleaning and collected between 6 and 25 samples per house with an average of 13.

Dead birds were removed from the houses at least once a day. The birds were disposed of using either burial (8.3%) or rendering (91.67%). All farms had manure removed by external contractors at the end of the production cycle.

4.4.1.2. Broiler layers

These farms receive birds from rearing farms. There were 20 responses from broiler layer farms. Two of the broiler layer farms were also table egg layer farms and another two were rearing farms.

None of the farms practiced moulting or was free range. All of the farms made use of nest boxes, manual egg collection and did on farm formaldehyde disinfection of eggs.

90% of the farms said they have been infected with SE before. All of these farms asked about the status of the birds received and would place birds from multiple grandparent flocks in one house. 89.47% of the 19 farms that responded to the question about the age of the newest house on the farm said that the house was older than 20 years.

Table 5 Broiler layer farm parameters

Factor	Responded	Min	Average	Max	Std. dev.
Personnel with full time employment	19	4	14.79	36	8.34
Casual personnel	18	0	4	6	2.05
Number of houses	19	4	7	20	3.36
Total surface area in meters squared of all houses	18	864	7756.27	28000	5779
Stocking density birds per m squared at full capacity	16	3.13	5.88	6.5	8.31
How many birds are on the farm when the farm is in full production?	17	9000	38826.47	65159	13553

Table 6 Broiler layer parameters

Factor	Responded	Min	Average	Max	Std. dev.
Number of times a day eggs are collected	20	4	4.05	5	0.22
Hours from collection until fumigation	18	0	1 hour	6 hours	-
*The longest period in **hours** from laying until the eggs are disinfected*	17	6	7.52	8	0.87
Chemicals used to disinfect the eggs	Either a mixture of Potassium permanganate and Formaldehyde or just formaldehyde are used in all farms for disinfection of the eggs				

The nest boxes were cleaned daily in the sense that the soft wood shavings were added if needed and if there was any manure it was removed daily. Six of the 20 farms reported washing the nest boxes weekly. The remaining 14 farms washed them at the end of the cycle.

Drinking water was filtered and treated with either chlorine dioxide or hypochlorite chips.

Manure was removed at the end of the cycle by external contractors for most of the farms. One farm had the manure removed and it went to a fruit farmer and another farm sent their manure to a cattle farmer on a monthly basis.

All the farms collected feed samples for Salmonella testing and made use of the same 3 feed companies as they were from the same larger farm groups.

Dead birds were removed from the houses at least once a day. The birds were disposed of using either burial (10%) or rendering (90%).

4.4.1.3. Hatcheries

There were seven hatcheries that participated in the study. Five completed the questionnaire. The hatcheries employed on average 14 staff members and produced on average 9,027,000 broiler chicks per year. None of the hatcheries reported having any casual staff. Of the five that responded when asked about the age of the hatchery, four were more than 20 years old and one was between five and ten years old.

Hatcheries receive eggs from between two and 15 farms; the majority received eggs from 15 farms.

All five hatcheries that responded share egg trays between farms. 75% of the hatcheries handle eggs from salmonella positive farms separately; 25% do not handle them separately.

Five hatcheries reported having a disease classification system in place for chicks. The disease classification included SE. Some were based on sentinel birds and boot swabs from the layer house. Others were based on testing of the hatching baskets.

All five hatcheries reported sampling and testing for Salmonella and Aspergillus.

4.4.1.4. Broiler farms

There were two arbitrary groupings of broiler farms. Small farms that had a capacity of up to 5000 broilers at one time and those that were able to house more than 5000 broilers at one time.

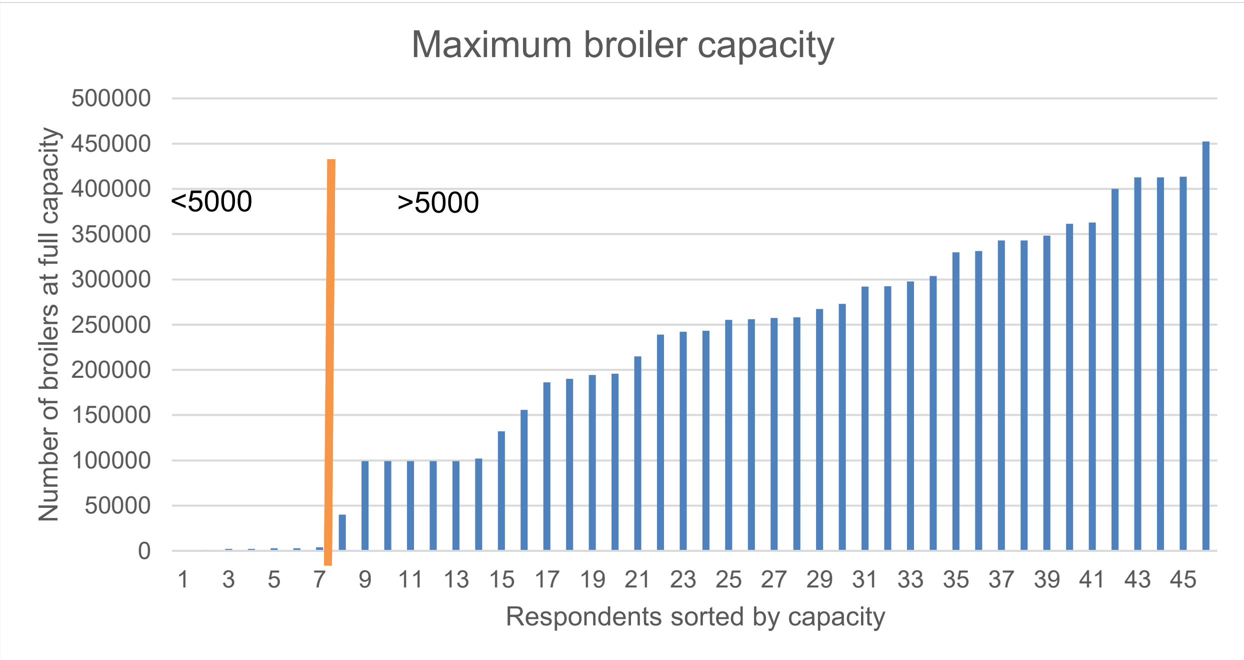

Figure 6 Broiler farms sorted by capacity

4.4.1.4.1. Small Broiler Farms

There were seven small farms that responded to the questionnaire. Four of the seven farms were sampled and all the test results were negative for *Salmonella spp*. All of them had the following in common: multiple flocks or poultry houses on the farm, existing staff do the cleaning of the houses, soft wood for used bedding, feed heat-treated feed on day one, none knew what vaccinations took place at the hatchery, none vaccinated for any poultry diseases, none collected any samples for bacteriology of feed, water or post cleaning. One farmer made use of a coccidiostatic medication containing nicarbazin and monensin. None of the farmers reported the use of any antimicrobials.

The farms did not make use of a veterinarian for their poultry. The farmers were asked when they would make use of a veterinarian and they indicated only if there was a big problem.

In the discussions, all of the small farmers indicated that they had seen the state appointed animal health technician who was doing surveillance for Highly Pathogenic Avian Influenza.

Table 7 Small broiler farmer parameters

Factor	**Responded**	**Min**	**Average**	**Max**	**Std. dev.**
Number of personnel with full time employment	7	1	3	8	2.31
Number of casual personnel	5	0	2.5	3	1.41
Number of houses	7	2	2.43	4	0.79
Total surface area in meters squared of all houses	7	60	249	428	155.34
Stocking density	7	6.67	10.34	21.78	5.3
How many birds are on the farm when the farm is in full production?	7	500	2271.43	4000	1184.22
How many hours before catching is feed withdrawn?	6	0	11	24	8.26

Table 8 Small broiler farm, oldest house age distribution

	< 5 years	**5-10**	**11-15**	**16-20**	**>20**	**Total**
Oldest house (number of houses)			1 (14.29%)	4 (57.14%)	2 (28.57%)	7

Table 9 Small broiler farm age distribution of the newest house

	<1	**1-2**	**>2-3**	**>3-4**	**>4-5**	**>5-10**	**>10-15**	**>15-20**	**> 20**	**Total**
Newest house number	3 (42.86 %)	0	0	2(28.57%)	1(14.29%)	0	0	0	1(14.29%)	7

There was not enough data provided to calculate the number of visitors for small farms. Small farms have very few visitors with contact to poultry.

Two of the small farms had the manure removed at the end of the production cycle by external contractors. One farm composted the manure in a field away from the house. The remaining four used the manure in the garden either directly or after composting. The farmers stored the manure 15 to 500m away from the poultry houses (average 207m).

Smaller broiler farms got their feed from various companies. Most of them were resellers and not from the manufacturer. Only two of the seven farms got directly from a feed company. Two of the farms were not sure if the feed company that supplied them had a certified GMP system in place.

Dead birds were removed from the houses at least once a day. The birds were disposed of using Incineration (14.29%), Burial (14.29%) Composting (14.29 %) or feeding to pig farms (57.14%)

4.4.1.4.2. Large broiler farms

These were broiler farms which are able to house more than 5000 birds when they are at full capacity. 46 large broiler farms participated in the survey. Of the 46 farms, 42 were sampled at abattoirs. For large broiler farms the questionnaire was completed at a head office responsible for the management of the farms that supply the abattoir. The broiler farms had 62-65% of houses older than 20 years (See tables 10 and 11) with 30 of the 40 farms having at least an annual veterinary visit.

Table 10 Large broiler farm, oldest house age distribution

	< 5 years	5-10	11-15	16-20	>20 years	Total
Oldest house (number of houses)	1(2.5%)	0	6 (15%)	7 (17.5%)	26 (65%)	40

Table 11 Large broiler farm age distribution of newest house

	<1	1-2	>2-3	>3-4	>4-5	>5-10	>10-15	>15-20	> 20	Total
Newest house number	4(2.5%)	0	0	0	0	1(2.5%)	5(12.5%)	5(12.5%)	25(62%)	40

Table 12 How often a veterinarian is used on large broiler farms

	Once a year	Quarterly	Every production cycle	Total
How often a veterinarian is used	7(17.5%)	26(65%)	6(15%)	30

The reasons given for use of a veterinarian were for health issues, general advice, production issues and routine visits.

When asked if the chickens were free-range there were 46 responses; 26.09% said yes and 67.39% said no. 6.52% had a mixed production system.

Cleaning teams, manure and dead bird removal companies, disinfectant suppliers and pest controller were the most common visitors respectively.

Manure was removed from the farms at the end of a production cycle for 33 of the farms (n=34). On one of the farms, the manure was composted 5km away from the farm and then returned after composting for use in vegetable gardens.

All of the large broiler farms reported that there are multiple flocks / houses on the farm, smaller birds culled using cervical dislocation, vaccinate for Newcastle disease and Infectious Bursal Disease, collect samples of water supply and test water for Escherichia coli and Faecal coliforms, chemicals used for cleaning houses are SABS approved for cleaning and disinfecting, samples for bacteriology collected after cleaning for verification of cleaning and soft wood shavings were used as bedding.

Table 13 Large broiler farms parameters

Factor	**Responded**	**Min**	**Average**	**Max**	**Std.dev.**
Number of personnel with full time employment	33	6	12.42	32	7
Number of casual personnel	34	0	3.33	6	1.44
Number of houses	46	3	11	25	4.70
Total surface area in meters squared of all houses	43	6000	19225	92589	17504
Stocking density	39	1.85	17.84	22.22	3
How many birds are on the farm when the farm is in full production?	39	40000	253704	452400	107054
How many hours before catching is feed withdrawn?	40	4	4.65	6	0.94

Doxycycline; Enrofloxacin, Trimethoprim Sulfadiazine and Kanamycin antibiotics were used in the last 12 months. Nicarbazin and Silinomycin Sodium were used for coccidia.

4.4.1.5. Abattoirs

There were five abattoirs that participated in the study. The abattoirs had the following in common: All of the abattoirs only slaughtered chickens and no other species of poultry. All abattoirs attribute a reference number to the flock slaughtered when the flock arrived. Ante-mortem inspection was done at all abattoirs. No chemicals were added to any of the scalding tanks. Scalding tanks were cleaned at least daily. The temperature of the scalding tanks ranged from 50 to 60 °C for four of the abattoirs. None of the abattoir raised the scalding bath temperature during breaks to reduce the microbial load of the water. All of them said it was not practical as they would have to wait for the baths to cool down again which would impact on production. A high scolding bath temperature would result discoloration of the chicken carcases.

None of the abattoirs apply, Acidified Sodium Chlorite (ASC), Tri Sodium Phosphate (TSP), Ozone, Sodium-hypochlorite, Calcium hypochlorite, Electrolytically generated hypochlorous acid, Citric acid, Lactic acid, Peracetic acid, Peroxy acids or Hydrogen, using sprays or dips following de-feathering or evisceration to disinfect the carcases. One abattoir applied chlorine at a concentration from 10-100ppm and two abattoirs applied Chlorine dioxide at a concentration of from 80- 300ppm following de-feathering. Four of the abattoirs apply chlorine at a concentration varying from 80 to 300ppm using sprays after evisceration. The One abattoir applies Chlorine dioxide to the carcass after evisceration but did not give the concentration used.

All the cleaning chemicals used were SABS approved for use in a food factory. None of them did cutting of imported meat and all of them only processed chickens that originated from their own slaughter lines within their own cutting plants.

None of the abattoirs applied any chemicals post chilling or applied crust freezing using continuous carbon dioxide belt freezing of skinless breast fillets to reduce salmonella on infected carcasses.

Table 14 Abattoir parameters

	Responded	Min	Average	Max	Std. Dev.
Carcasses (Number)	4	150000	3287500	9000000	3920538.87
Meat (Metric tons)	4	200	973578	3700000	1819448.88
Number of personnel with full time employment	3	15	195	290	155.96
Number of casual personnel	3	0	22.5	30	15
Maximum daily through put	Not applicable	850	73970	320000	137916.567
Number of people at evisceration	5	3	5	20	7.28
Cutting plant temperature	5	8	10.8	12	1.7

Packaging of the product was dependant on the type of product as well as the brand and not all products were packed in leak proof packaging. Absorbent material was not placed in all packaging as this was also dependent on the product. Product labelling contained details about safe storage and shelf life but no details of potential inherent risks contained in the product. Product labelling was also dependent on the brand and type of product.

One of the five abattoirs had a process that was followed should there be additional risk for salmonella. The other four did not have any prepared processes in place. The procedure followed was blast freezing to less than -12°C. Time below -12°C was not specifically measure or managed.

4.4.1.6. Social network analysis

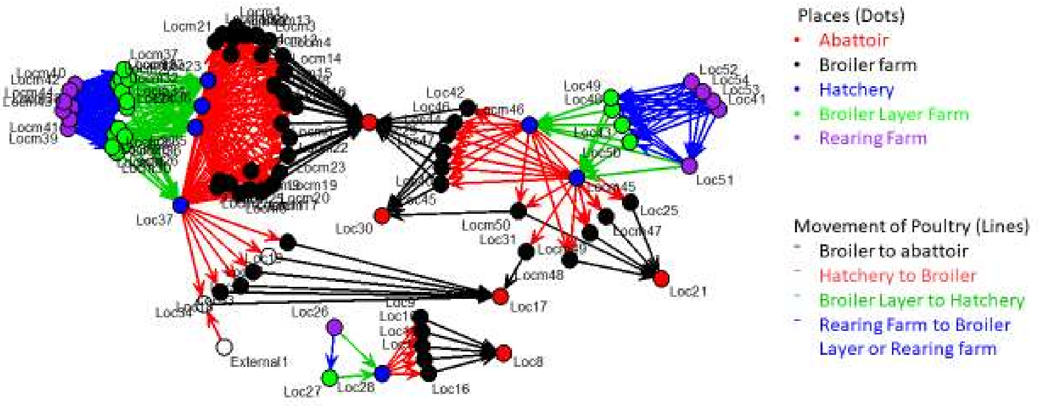

Figure 7 Graph of survey participants and relationship /movement of broilers and eggs between them

In Figure 7 the network is a directed network, nodes have both indegree (connection coming in) and outdegree (connections going out) centrality scores. Centrality measures how central or well connected a node is in a network.

In Figure 7 the 94 nodes (Dots) are the farms, hatcheries or abattoirs and the 381 edges (lines) represent the movement of broilers or eggs between them. The flow of the edges are unidirectional and unweighted. The level of data collected in the survey did not allow for the weighting of edges between nodes. There were 3 main subnetworks. The top left one with lots rearing farms, broiler layer farms and lots of broilers farms all dedicated almost to one abattoir, one hatchery attracting broiler layer farms that are also connecter to other hatchery that was dedicated to another abattoir, a subnetwork on the top right connecting to 4 abattoirs to two hatcheries with one of the 4 abattoirs connected to the top left subnetwork and a subnetwork at the bottom of Figure 7 that is a standalone subnetwork.

Table 15 The amount of indegrees per node

Number of indegree	**0**	**1**	**2**	**4**	**5**	**7**	**13**	**33**
Number of nodes	13	12	13	28	8	15	4	1

Table 16 The amount of outdegrees per node

Number of outdegree	**0**	**1**	**2**	**4**	**5**	**6**	**11**	**12**	**13**	**27**	**33**
Number of nodes	5	45	12	13	5	1	1	1	7	3	1

Looking at Table 15 and Table 16 both in and out degrees we can see that degrees centrality for some nodes is very high; making them important nodes in the network due to the amount of connections they have. The abattoirs had only outdegrees and the rearing farms only had indegrees.

The proportion of direct ties in a network relative to the total number possible is called the density of the network which is 0.043.

A dyad is defined as any pair of nodes. In a directed network, a dyad is said to have a reciprocal relationship if there exists an edge from one node to another and an edge back again.

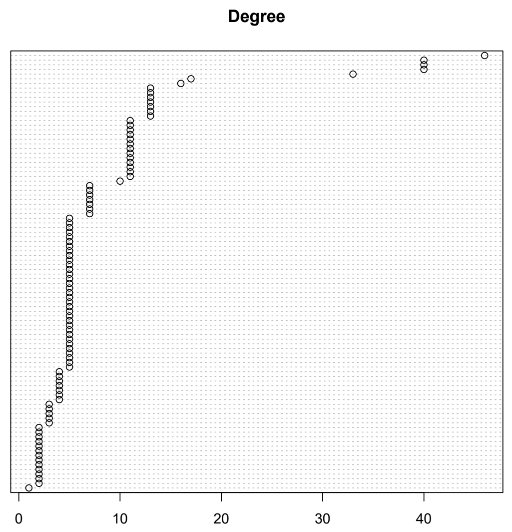

Figure 8 Degrees centrality per node

The dyadic reciprocity of a graph is the proportion of dyads which are symmetric: 0.91

Transitivity is a triadic, algebric structural constraint. In its weak form, the transitive constraint corresponds to *a->b->c => a->c*. The fraction of potentially intransitive triads obeying the weak condition = 0.011

If we compared this results to a simulation of random graphs of the same dimension and same density we can see that our transitivity is lower than 95% of the transitivity of the random graphs. This means that the network is not a random network.

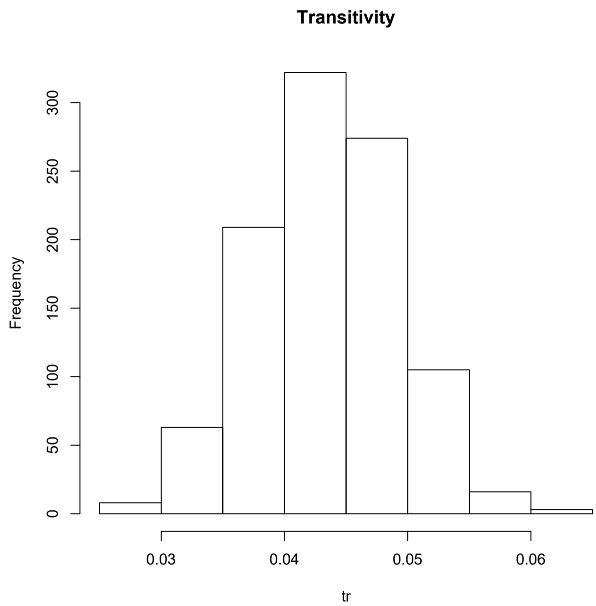

Figure 10 Transitivity of a simulation of random graphs of the same dimension and density

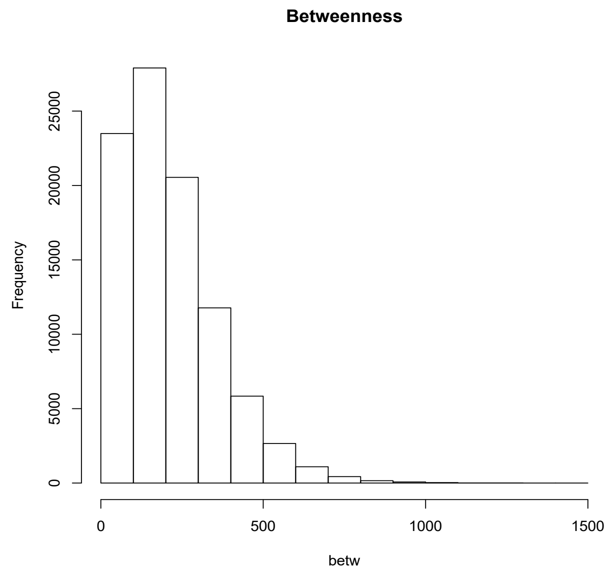

Figure 9 Betweenness of a simulation of random graphs with the same dimension and density

Connectedness=0,825 Proportion of dyads for which there exists an undirected path

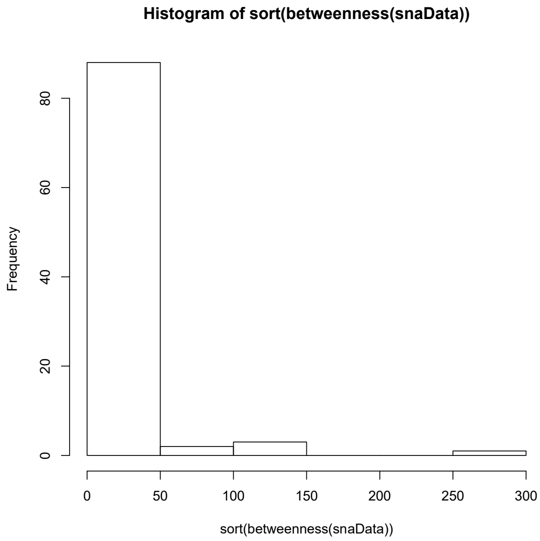

Figure 11 Betweenness of SNA from data

Betweenness is given for each node: Conceptually, high-betweenness vertices lie on a large number of non-redundant shortest paths between other vertices; they can thus be thought of as "bridges" or "boundary spanners."

The mean is 13.76. Our mean is lower than 95% of the betweenness of the random graphs of the same dimension and density. This means that we have lots of nodes (particularly farms) with very low betweenness and few nodes (especially abattoirs but also hatcheries) having a high betweenness.

Table 17 Table of K-cores

K-cores:	1	2	3	4	5	11
Number of nodes:	1	17	6	7	39	24

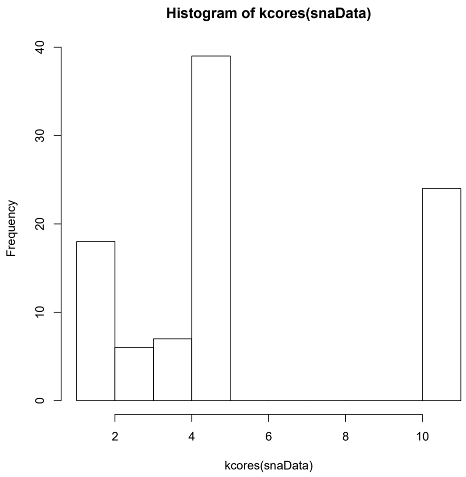

Figure 13 Histogram of kcores SNA data

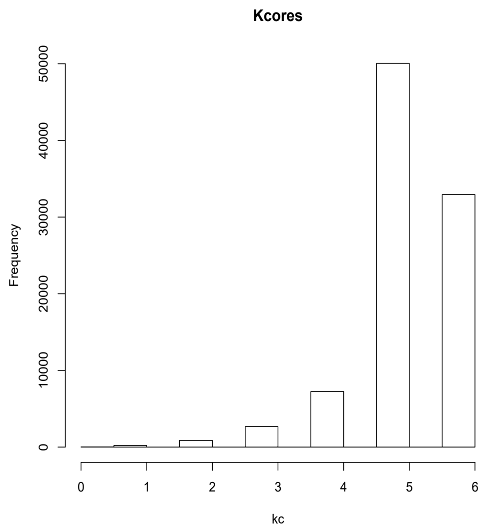

Figure 12 Histogram of kcores of a simulation of random graphs with the same dimension and density

If we compare to random networks we see that k-cores do not exceed 6 in the random network. Whereas in our case we have almost 25% of our nodes that have a k-core of 11. This means that almost 25 % of our nodes can lose 10 edges before they are no longer connected. Some nodes are well connected to each other and therefore more important. The nodes with the highest k cores were the hatcheries as they connected to broiler farms and layer farms.

4.4.1.7. Bacteriology results

499 samples were collected from 5 abattoirs. For each sample, farm details were provided. For most samples the number of birds in the house, hatchery, broiler layer and broiler layer rearing farm identification were provided.

Table 18 Abattoir sample breakdown

	Total	Min	Average	Max	Std. Dev.
Number of samples collected per abattoir	499	30	99.6	297	111.41
Number positive	14	0	2.8	13	5.7
Number negative	485	30	96.2	280	103.83
Number of birds in the house where the samples came from	844542 (41 farms)	20067	2150696.5	7862509	3819757.37
Number of separate days when samples were taken	44	2	8.8	27	10.35

Table 19 Abattoir time and sampling details

Abattoir- ID	Number of samples	Number positive	Number negative	Maximum daily through put	Separate sampling days	Total Number of birds in the houses where the samples came from
Loc8	70	1	69	25000	7	672210
Loc29	297	13	280	320000	27	7862509
Loc17	59	0	59	850	2	20067
Loc21	43	0	43	4000	5	48000
Loc30	30	0	30	20000	3	-

Of the 17 samples tested by serotyping at the Onderstepoort Veterinary Institute 14 (2.81% of the total samples collected) were serotyped as *Salmonella enterica subsp. enterica.* The other 3 samples were negative for Salmonella spp.. Serotypes *S.Heidelberg*, *S.Muenchen* were isolated together from one farm.

Serotype *S. Infantis* was isolated from two completely separate abattoirs and producers. The one abattoir took their own samples at the same time and their sample was positive for salmonella at a separate laboratory. Therefore, it is unlikely that this serotype was cross contamination at the lab. Further investigation is needed, to draw a conclusion as to the origin of this serotype.

9 (20%) of the 46 farms were positive for Salmonella spp.. If a farm was positive for salmonella on average 16% (10-30%) of the samples collected from that farm were positive.

Table 20 Salmonella serotypes

Serotype	Number of farms ISOLATED from	Number of isolates
S. Hadar	2	3
S.Heidelberg	1	1
S. Infantis	2	2
S.Muenchen	3	4
S. Newport	1	3
S. Anatum	1	1

4.4.1. Antibiotic usage

The use of antibiotics affects the microbial composition of the gut in poultry. Antibiotics used also gives an indication of selective pressure on the bacterial population for resistance to specific antibiotics. The farms were asked to provide the trade names and the active ingredients use in any treatments or growth promoters that the poultry were given in the last 12 months. 55 of the 91 farms reported to have used antibiotics in the last 12 months.

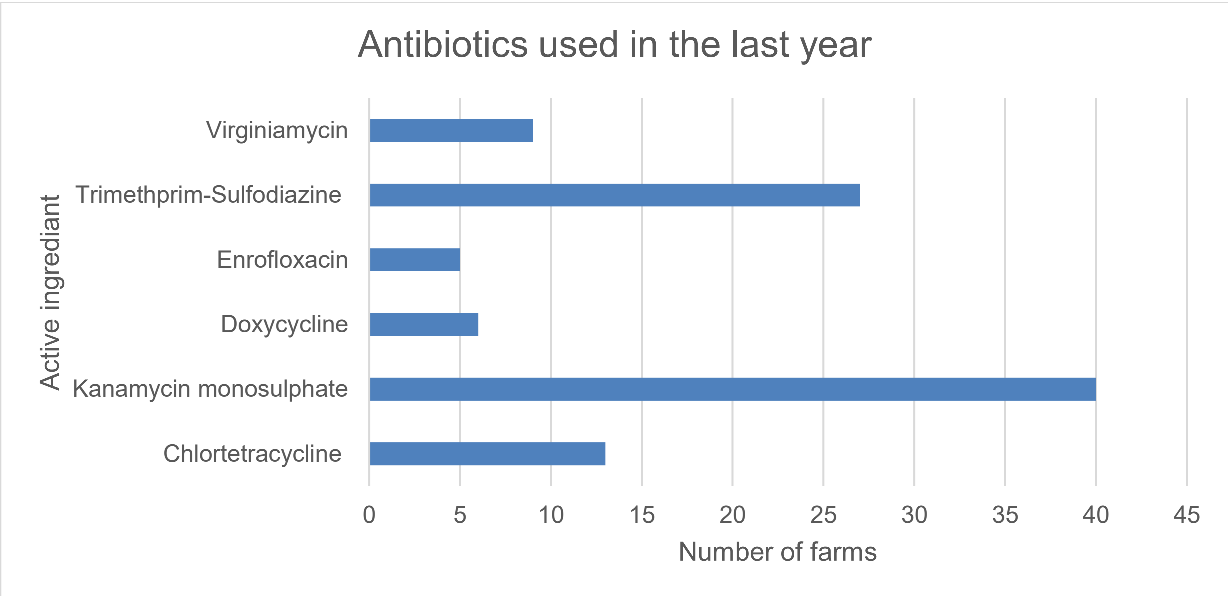

Figure 14 Antibiotic used on farms in the last 12 months

4.5. **Risk characterisation (2): Data analysis**

4.5.1. Questionnaire and bacteriology analysis

In this section of the analysis, only data of the broiler farms sampled at abattoirs were considered.

For variables that had the same response for all the respondents there was no further analysis done. For the remainder of the variables odds ratio and chi squared tests were conducted where the outcome of the variable was looked at against the likelihood of the farm having tested positive for *Salmonella.* No statistically significant outcome was seen when the following were assessed using odds ratio (OR):

Table 21 Factors that did not have a statistically significant outcome on bacteriology when using odds ratio

Factor	**Respondents that were tested and responded**
Who does the main cleaning?	40
Where do you source the drinking water for the birds?	40
Number of houses - above average	46
Is mash feed given instead of granular feed (heat-treated) on day 1?	40
How many hatcheries supplied the farm in the last year if more than 1	40
Have you ever been informed by the hatchery that the chicks could come from a farm potentially infected with Salmonella?	40
Has the farm been previously infected with SE?	40
other medication used in the last 12 months?	40
Coccidiosis preventive medicines used in the last 12 months?	40
Anti-parasitisides (mites, worms) used in the last 12 months?	40
antibiotics used in the last 12 months?	40
Do you ask what the disease status of the farm that supplies chicks to you is?	45
Broiler number more than mean	46
Are the houses that are being partially depopulated always caught before those that are fully depopulated populated on the same day?	39
Are the chickens' free range?	43
Are samples taken when chicks arrive at the farm?	34
Are any of the houses accessible to wild birds?	40
How old is the establishment?	46
Source of drinking water	46

Table 22 Factors that were statistically significant using univariate analysis (OR)

Factor	Code	Respondents that were tested and responded	OR	P-Value
Organic acids in the feed or water?- Yes	MP1[SQ8]	39	9	0.01235
Above average- Total surface area in meters squared of all houses		46	4.714	0.0718
Above average number of personnel with full time employment		46	2.8	0.2
Above average number of casual personnel		46	8.75	0.051
Post cleaning bacteriology for Salmonella spp.	CP9[SQ3]	40	9.333	0.010
Post cleaning bacteriology for Faecal coliforms	CP9[SQ2]	40	9.333	0.010
Probiotics in the poultry feed	MP1[SQ9]	46	0.39	0.227
Are you willing to accept chicks / eggs from a breeder where birds were previously diagnosed as positive for Salmonella enteritidis?		40	6	0.305
How old is the newest house on the farm? - more than 15 years old		40	5.2	0.111
Slaughtered at a abattoir that uses mechanical evisceration		46	-	0.14
Abattoir Loc29		46	6.095	0.0732
Producer company L		46	11.33	0.0042
Specific layer farm - Locm36		41	4.5	0.06
More than 1 hatcheries supplied the farm for the specific sample.		40	8.75	0.0510

4.5.1.1. Principal Components Analysis

Figure 15 Variables factor map (PCA) Dim 2 and 4 best represents farm positivity with Dim 2 and 4 representing statistically significant correlation to farm positivity of 0.52 and 0.54 respectively and a 23.13% variance. Data tables for the PCA are available on page 73

From the PCA the factor reduction resulted including Loc23, Locm36, “More than one hatcher“, “surface area” Full time staff larger than mean “Full time staff larger”, the use of probiotics “MP1_SQ9”, accepting birds from a previously infected farm “SQA1_SQ2” and “New house 15 years or more “ in logistic regression as there was a correlation with Dim 2 and Positive. “Surface area”, Loc23, “MP1_SQ8” the use of organic acids in feed and locm36 were included since there was a correlation with Dim 4 and Positive. In Figure 16 Individuals factor map (PCA) Dim 2 and 4 there is an even distribution of the farms.

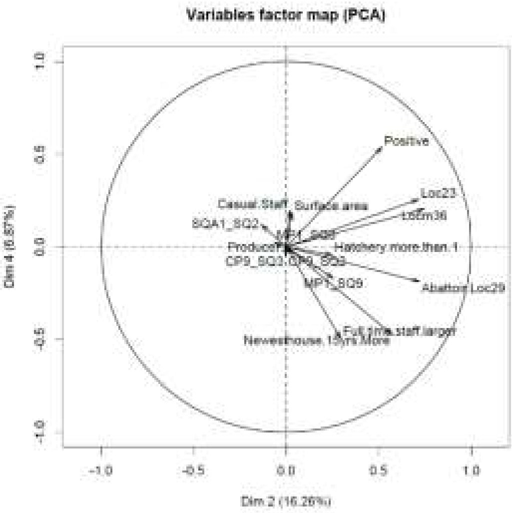

Figure 15 Variables factor map (PCA) Dim 2 and 4

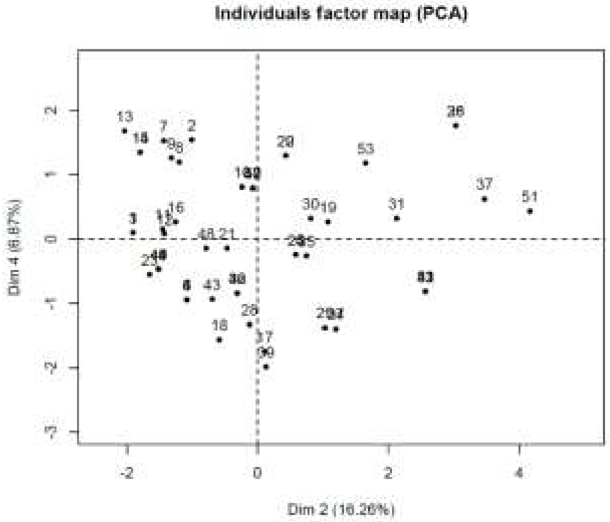

Figure 16 Individuals factor map (PCA) Dim 2 and 4

4.5.1.2. Logistic regression and goodness of fit test

Table 23 logistic regression results for Locm36

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)		
(Intercept)	-1.7228	0.4855	-3.549	0.000387	***	
Locm36	1.7228	0.8577	2.009	0.044584	*	
Significance. codes:	0 '***'	0.001 '**'	0.01'*'	0.05'.'	0.1''	1
AIC	43.162					

The odds ratio from the general linear matrix was 5.6. CI is [1.04-30.08] 95% calculated from the coefficient of the general linear matrix results and the standard error. The significant factor remaining from logistic regression was Locm36 which was a broiler layer farm that supplied eggs to hatcheries who then in supplied chicks to some of the farms that were sampled.

Hosmer and Lemeshow goodness of fit (GOF) test was performed and the results showed a p-value higher than 0.05, which signed an absence of difference between fitted data and observed data and the binary data collected therefore, the model is a good predictor of a positive outcome in this sub population

4.5.1.3. Historical data analysis

A Ripley's K value analysis was done on the historical data. The D0plot can be seen in Figure 20. The statistical test gave a p-value of 0,039 which means that there is a statistically significant spatial-temporal cluster. There were clusters over time and space.

In Figure 18, a spatial map of the data relative to a point illustrates how far the locations, that reported SE, are from each other.

Figure 17 : Residue plot, is a plot of the standardised residuals $(\widehat{K}_{st}(s,t) - \widehat{K}_s(s)\widehat{K}_t(t)))/SE(\widehat{K}_{st}(s,t))$ against the product of the space and time K functions $\widehat{K}_s(s)\widehat{K}_t(t)$. Values of more than two above or below 0 are indicative of a space-time interaction occurring. This graphs shows that there is space time clustering.

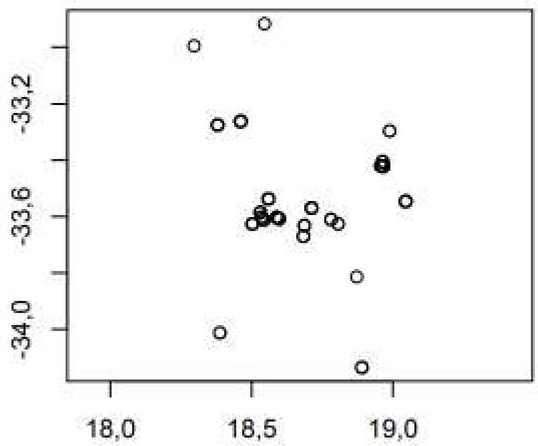

Figure 18: Data map of location relative to a point

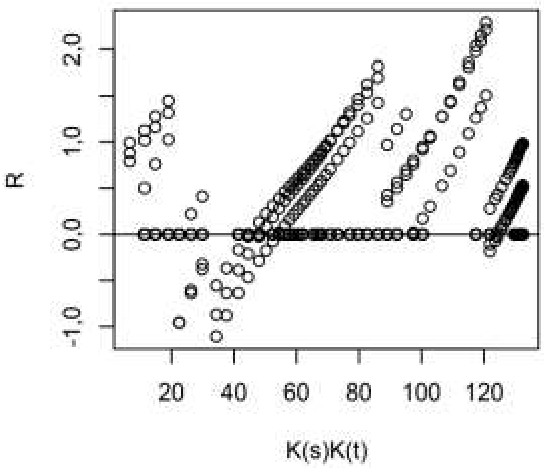

Figure 17 : Residue plot

Figure 18, is the physical location of places that reported SE during the period analysed relative to a geographic point.

Figure 19, is a perspective graph of the difference between the space-time K-function and the product of the special and temporal K-functions. A cluster close in space and time (the peak between 0-5 months) and within a distance of 0.5 degrees. All the peaks on the time line are always within a very close distance meaning that distance maybe a very important factor in terms of disease spreading.

Figure 20, summarizes in a histogram of the test statistics (p-value) from a Monte Carlo simulation. The value of p-value is indicated by the vertical line at 0.11. Meaning that the result is significant.

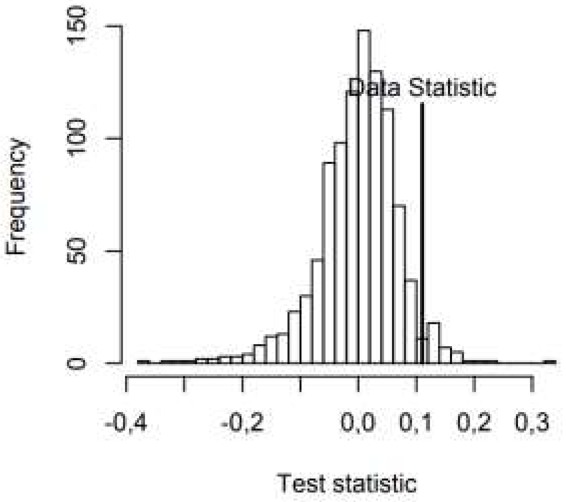

Figure 20: Monte Carlo simulation results

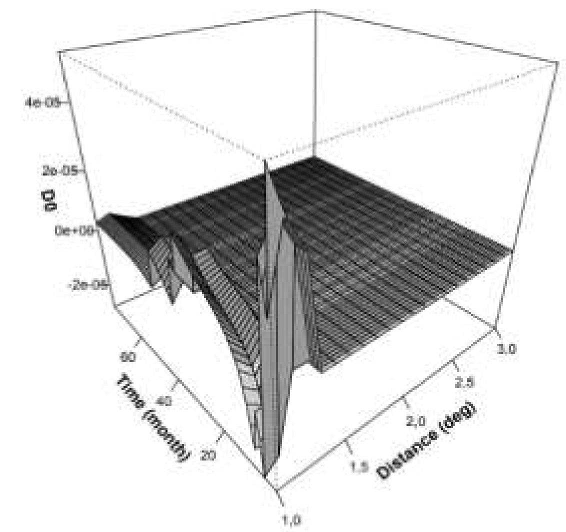

Figure 19: D0 as a function of time and distance for SE in the Western Cape

Chapter 5: **Discussion**

5.1. Risk Characterization (3)

Given that the three leading single causes of premature mortality in South Africa are HIV-related conditions, TB and pneumonia (Groenewald *et al.*, 2017), salmonellosis caused by SE is likely to cause more severe symptoms in South Africa than what would be expected in an immunocompetent population. Therefore, additional care is required from the poultry food chain in South Africa compared to poultry food chains elsewhere.

SE exposure from poultry originating from the Western Cape is likely to be low due to the low detection rate of SE at abattoirs.

5.2. Design of the questionnaire

A large part of the study for risk characterization was the design and implementation of the questionnaire. Therefore, it is prudent to highlight some of the pitfalls of the questionnaire design and implementation so that future studies can benefit.

Access to farmer managers for questionnaire completion was a challenge in larger companies. Some companies preferred to respond on behalf of the farms they are responsible for. The person answering the questions at the company was also the person responsible for the health policies the farms need to abide by and therefore may have been more inclined to give answers based on company biosecurity policy rather than the reality on the farm. Having a single person responsible for multiple responses to the questionnaire led to questionnaire fatigue.

In South Africa, language used among farmers and the terminology farmers use for the different stages of broiler farming is not standardised. In the interviews with the farmers and abattoirs, the terminology had to first be clarified before proceeding with the questionnaire administration. Terminology in the abattoir environment was much more standardised.

The question regarding total broilers produced and total manure produced in one year was not understood correctly by all respondents, and the data had to therefore be removed from the results.

The question dealing with staff living on other poultry farms or staying with family members working on other farms was not easy for the farmers to answer. They were largely unsure of this information. Their corporate policy may state that staff may not stay on other farms but they did not have any procedures to check this or specific recourse if it was discovered that a staff member stayed on another farm.

When looking at the age of a farm versus the age of the infrastructure on the broiler farms, 13 out of 46 farms had poultry houses on them that were older than the farm itself. In the results, the age of the oldest poultry house was used as the infrastructure age and not the age of the farm. Previous studies that identified the age of the farm as a risk factor did not mention this issue in terms of the interpretation of the survey results.

The question regarding who did the main cleaning of the poultry houses or facilities was hard to interpret. Farmers had day to day staff that did the daily cleaning. Abattoirs had staff who did cleaning as production continues. It had to be explained to most respondents that the question was referring to deep cleaning.

In South Africa, some antibiotics are registered as growth promoters. This caused confusion with the farmers as they were not aware that these were also antibiotics. Farmers had to be asked to clarify what the active ingredients were.

The question about how soon after collection eggs are disinfected caused confusion, as the farmers were not sure if fumigation with formaldehyde counted as disinfection or not. Clarification that fumigation with formaldehyde was also a disinfection had to be given.

The question dealing with nest box cleaning was not worded well. Farmers reported that they removed droppings daily and were not sure what the meaning of cleaning was. The question did not allow for enough detail. One of the farmers explained that the scrape the egg boxes and add formaldehyde to them at specific intervals during the cycle. A more open-ended question would have been appropriate.

That 75% of hatcheries handle eggs from salmonella infected farms separately is unlikely as they are not designed to be able to do this. Generally setters consist of one big room which is filled before being turned on. It is unlikely that they would put a half-full setter on. More investigation is needed to fully understand how the hatcheries operate and what they do to prevent the spread of Salmonella.

5.3. Spatio-temporal analysis and social network analysis

As Salmonella can come from environmental sources, it is important to see if there is a link between regions of the outbreaks and the number of outbreaks. It is also important to see if there is any seasonality to the outbreaks. One would expect to see more reports of disease closer to pathogen sources, and fewer reports in areas far away from these sources.

These patterns are complicated by transmission that can occur from environmental reservoirs, previously infected staff members, pests and poorly cleaned houses. Reflecting the dynamics of transmission, human behaviour and environmental variability is a challenge due to the variability of the spread of salmonella.

This study did not find SE in any of the samples collected. However, historical data shows that SE is present at the farm level. There are a number of potential reasons for this. The historical data is from different sampling points, not only abattoirs, and survivability of SE may be less than other *Salmonella enterica* serotypes in abattoir samples, no literature was found supporting this notion, the farms may have improved control of SE and SE may have gone undetected in the study due to sample sizes. The presence of other pathogenic salmonella indicates that there is room to improve *Salmonella* subspecies *enterica* control.

The spatio-temporal analysis showed a significant clustering consistent with what Ansari et al. (2017) found in Iran. The spatio-temporal clustering may support the notion postulated by Ansari et al. that when there are several farms with high poultry density near to each other, the chance of salmonella contamination may increase. However clustering could be explained by grouping of farms that cooperate under a single company or as a cooperative. These spatio-temporal clusters could be as a result of a supply chain created by single company or cooperative ensuring single origin of feed, single service provider, or single supplier of chicken or eggs used though the company or cooperative. Clustering in this case may not be as a result of a disease factor but the nature of the production system instead. Figure 18 is a graph of the locations of the historically reported cases, showing clustering that is geographic. It makes logical sense that farms be located closer each other, their service providers and infrastructure, when they are all managed together. In an article by Diego et al. (2010) they corrected the spatio-temporal clustering of cholera based on heterogeneity of family grouping and found that spatio-temporal trends were not significant. Raised incidence modelling that takes into account the cases but also the location of the non-cases as well as farm grouping should have been used. Unfortunately, information about testing where the results were negative as well as the linking of outbreaks to groups of cooperating farms was not in the historical data. Farms in the Western Cape mostly operate as groups either through co-operation with each other and the abattoir they supply or by single company ownership or contractual obligation to one company. The official data collection of diseases which may have a spatio-temporal link should include elements of the production system within the reporting with the main element being the cooperative farming group or parent company and not only the location of the outbreak.

According to the social network analysis, there are edges between production chains which could enable spread of an organism like salmonella between production chains.

There were a few nodes with a high degree of centrality. A significant odds ratios for a specific abattoir and broiler layer farm, which are also locations with a high degree of centrality, supports the notion that these locations are places where the disease is likely to be detected and possible locations where the disease can be shared between farmer groups. If farmers are not able to apply strict bio-confinement and hygiene measures, the groups of farmers or companies should decrease the centrality of some of the nodes in order to reduce the risk of spreading the bacteria. This will decrease risks of other diseases as well. Social network analysis could be used as an affordable tool to redefine sampling sites and improve surveillance for diseases in resource poor countries.

5.4. Pathway

Grandparent farms (who supply chicks to rearing farms) were not included in the study as there were not located within the sampling area. Future studies on the grandparent farms should be done to assess the risk they present to the broiler meat chain in terms of salmonellosis in humans.

The age structure of hatcheries (80% >20 years), rearing farms (83.33% >20 years), broiler layer farms (89.47% >20 years) and broiler farms (62% >20 years) may present a risk for diseases. This concurs with what was found by Van Hoorebeke et al. (2011) that due to the age of houses there are likely to be structural wear, outdated technology, biofilms and build-up of pathogens on the farms.

The age of the newest broiler house being older than 15 years was identified as a significant risk factor of the broiler farms in this study.

None of the farms monitored dust or had any formal dust control program in place even if they were in close proximity to dirt roads. This is similar to the findings of Humhrey et al., 1992 and Holt et al. 1998. This was not found to be a risk factor in this study.

This study found that broiler farms that were willing to accept eggs or chicks from a breeder where birds were previously diagnosed as positive for SE were more likely to have a sample detected as positive for salmonella at the abattoir. Guard-Petter, (2001) Øystein et al. (1996), Christensen et al. (1997), Davies et al. (1997), and Rose et al.(1999) had similar findings. 57% of smaller broiler farms did not ask the status of the chicks received and this would increase their risk. When 90% of the broiler layers were previously infected, it is hard for a farmer to reject eggs or chicks from a broiler layer that was previously infected.

It was surprising that none of the rearing farms reported having had SE before when 90% of the broiler layers reported been diagnosed before. To get more detailed information on the rearing farms and the effect of the documented risk factors on the farms, a more detailed analysis and sampling of farms is needed. The author suspects that the greatest risk factor to rearing farms is their aged infrastructure more detailed investigation of these farms would be warranted given the average age of the farms is over 20 years.

There were 29 small farms in the WCDoA farm list. Of the seven farms that completed the questionnaire, none were on the list. In the discussions with the farmers, most of them did not make use of a veterinarian at all unless there was a disease outbreak with mortalities that were very high. Most of them did not belong to the poultry association so therefore would not have known about the survey being conducted in response to the highly pathogenic avian influenza.

57% of the small farms made use of pig farms for dead bird disposal. The use of pig farms for can lead to infection of the pork meat chain with salmonella.

Due to the pathway of salmonella involving the transmission of salmonella from faeces, manure management should be one of the most important factors for controlling Salmonella on an infected farm. The manure stored on the farm can act as a source for recontamination of new chickens brought onto the farm. It also acts as an attractant to pests who could carry other diseases. The small broiler farms generally did not manage manure safely and this represents an increased risk to them as well as those farming around them. For the large farms, more detailed investigations into manure management is needed. The method of transport, covering of manure during transport and disinfection of vehicles transporting manure was not covered by the scope of the survey.

Small farms had younger houses than the large farms. The lack of vaccination, testing of water, feed testing and testing of birds for salmonella did not influence the level of salmonella detected at abattoir level for these farms.

All of the samples collected from small farms were collected at an abattoir that used manual evisceration which was found to have a protective effect on Salmonella detection. The author suspects that the salmonella may have been effectively removed by carcass washing and good evisceration. Hue et. al. (2011) identified mechanical evisceration as a risk factor for SE detection and this study found the same effect.

If a farm that was positive for salmonella, 10 to 30% (average 16%) of the samples were positive. It is likely that proper slaughter, hygiene, carcass rinsing and freezing of the samples reduce the number of positive samples from an infected farm significantly (Codex Alimentarius Commission, 2011). It is therefore also likely that a farm that has a low infection rate or chicken carcases with a low contamination rate may not have been detected with the sampling and sample handling methods used in this study. The aim of this study was not to establish a farm prevalence for salmonella but to understand the risk of presence of salmonella after slaughter at an abattoir, taking the abattoir slaughter and hygiene methods into consideration.

Probiotics in the poultry feed was a protective factor which was most likely due to competitive exclusion as discussed by Guard-Petter, 2001. But this may also have been a confounding factor as groups of farms identified as risk factors did not make use of probiotics. More data would be required for confirmation of the effect of probiotics.

The use of organic acids was found to be a significant risk factor using OR. This is in opposition to what Hue et al. 2011 found. It does not make biological sense for organic acids to be a causal factor for positive salmonella culture at abattoirs. It is suspected that farms that know they are infected are trying to use organic acids to reduce the amount of salmonella faecal shedding at farm level. It is also suspected that these farms do not use the acids as a routine. Further investigation on the use of organic acids on chicken farms in the Western Cape is needed.

Post cleaning bacteriology testing for Salmonella and faecal coliforms were identified as risk factors. These are confounding results as producer company L, was one of the larger groups of farms, which was also recognised as a significant risk factor. Producer L is the only large producer that tests for the above bacteria after cleaning.

Above average surface area (Floor size) of houses and above average number of fulltime personnel were both identified as risk factors using OR. These are likely to be confounding factors related to the size of the farms. Size of farm is related to having more than 3 houses which was identified as a risk factor by Øystein et al. (1996).

Above average number of casual staff was identified as a significant risk factor using OR. Casual staff are not likely to be trained as well and to not be as careful about hygiene procedures that are in place. This may be a unique risk factor for developing countries. It is also possibly an indication of weaknesses management planning.

Where a farm gave more than one hatchery as the supplier of the chicks that were sampled at the abattoir, there was a significant increase in risk that those carcases would be positive for salmonella. More than one hatchery means an increase in the opportunities for salmonella to enter the farm, as there are likely to be more layer farms in the supply of the eggs to the two hatcheries.

There was not enough sampling data available to compare the processes at abattoirs with each other as some abattoirs did not have enough samples collected from them. An exception was the method of evisceration discussed above.

Routine salmonella samples collected by abattoirs were all taken at the start or middle of a production shift. They could enhance their ability to detect salmonella if their samples are instead collected late in the production shift. Systematic washing of all carcasses was normal in all abattoirs.

When abattoirs knew of an increase in risk of salmonella due to the farm of origin or sampling results of the farms of origin they did not have adequate procedures in place to address the increase in the risk to the consumer.

Abattoir product labelling did not indicate that there were inherent risks to the product. Consumer education could be enhanced by improving information contained on the labels used.

Decontamination of the outside of packaging was not done at any of the abattoirs. The packaging material does not support microbial growth but, given that salmonella is able to survive in harsh conditions and cool temperatures, the outer packaging may present a risk factor for human infection. Further studies are needed to evaluate the extent of the impact on consumer health.

5.5. Bacteriology results

In the study by Zirshiri (2016), 51% of the caecal samples were positive for salmonella. The caecal samples would not take into account the processing at the abattoir and would thus be expected to have a higher salmonella content.

5.6. Antibiotic usage

Enrofloxacin, doxycycline, Virginiamycin, chlortetracycline, kanamycin monosulphate and trimethoprim-sulfadiazine were used as antibiotics, for prevention or control of disease, or growth promoters in poultry of the Western Cape according to this study. Zirishiri (2016) showed that there was resistance to chlortetracycline, kanamycin monosulphate and trimethoprim-sulfadiazine which were used in the farms surveyed in this study.

Virginiamycin is one of the streptogramins which are important in human medicine. It has been shown in other countries that virginiamycin use can result in a population of quinupristin-dalfopristin-resistant *Enterococcus faecium* isolates (Hayes, McIntosh, Qaiyumi, Johnson, English, Carr, Wagner & Joseph, 2001). Enrofloxacin, doxycycline and virginiamycin were not found in any study conducted in the South African broiler production chain.

The movement of manure to composting and other food systems could spread resistant bacteria to other food chains and this movement warrant further study.

This study did not have additional funding for antimicrobial sensitivity testing. Future studies should be conducted in the Western Cape with a specific focus on sensitivity antimicrobials used in the industry and sensitivity to the classes of antimicrobials they originate from.

5.7. Risk factors confirmation

The use of PCA helped remove most of the confounding factors, before logistic regression was performed. It results from the logistic regression that the only risk factor identified was Layer farm Locm36. Layer farm Locm36 is a broiler layer farm in the chain. Locm36 was not sampled as it was a supplier of eggs to hatcheries that in turn supplied chicks to broiler farms. 8 of the broiler farms indicated that layer farm Locm36 supplied the eggs to the hatchery for the chicks that they received. But Locm36 could be an integrator factor as the eight farms which received chicks from Locm36 had a newest house that was older than 15 years, "SQA1_Q2" were willing to receive chicks from previously infected farms, had above average number of houses, received chicks from Loc23 and were slaughtered at Loc29. Five of the farms had above average full time staff. All of these risk factors were found to be significant when using univariate analysis and most of which correlated to Dim2 or Dim4 in the PCA. Locm36 may just represent the best aggregate for these risk factors.

Chapter 6: **Conclusion**

The risk of SE in the Western Cape Province of South Africa was analysed by describing the risk pathway, highlighting the critical points (where the risk may be introduced or may increase drastically) of SE through the food chain. Questionnaires should be designed for each stage of production taking company information management systems into account to minimised respondent fatigue. Risk assessment is a tool that can be applied when empirical data is limited.

Social network analysis is a useful tool that can be conducted with minimal data and it provides a large amount of value in determining likely sampling sites and places of increased risk. PCA is a helpful tool to summarize the risk factors but its results need to be considered in context of the data and univariate analysis. The most likely risk factors in the Western Cape context for salmonella are the age and size of the farm. Spatio-temporal analysis should only be done on data when interactions between producers are known or it will not be possible to interpret the results accurately. Official data recording of poultry diseases should include elements of the production systems such as farmer cooperatives, parent company identification and backward traceability.

Additional data is needed at each production stage and more details as to why age and size are risk factors is needed. Further clarification of consumer habits such as purchasing of brine injected frozen poultry meat, transport and preparation of poultry meat and the proportion of the vulnerable population is needed. Distribution, secondary processing, retail and consumer characteristics were not part of this study and therefore more work is needed to develop a final overview of risk.

The risk of SE exposure from broiler meat originating from the Western Cape is low, however historical data shows that it is present in the population. In addition, a number of non-typhoidal *Salmonella subspecies enterica* were isolated during the study. Given the high rates of immunosuppressive diseases within the local population, it is important for broiler producers to be aware of the zoonotic potential of non-typhoidal Salmonella (including SE) and continually improve their control measures through policy revision, education of role players in the production system and implementation of internationally recognised control practices.

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Appendix A. Animal Ethic approval certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**A risk assessment of *Salmonella enteritidis* in the Western Cape, South Africa**
PROJECT NUMBER	**V076-17**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. V Henwood**

STUDENT NUMBER (where applicable)	**U_04222067**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Gallus gallus domesticus**	
NUMBER OF ANIMALS	**400**	
Approval period to use animals for research/testing purposes		**July 2017- July 2018**
SUPERVISOR	**Dr. E Etter**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 31 July 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15

Appendix B. Data

General risk factors of importance, Suppliers of birds or eggs as well as visitors

Rearing farms

Table 24 Rearing farms oldest house

	< 5 years	5-10	11-15	16-20	>20	Total
Oldest house (number of houses)		2 (16.67%)			10(83.33%)	12

Table 25 Rearing farms newest house

	<1	1-2	>2-3	>3-4	>4-5	>5-10	>10-15	>15-20	> 20	Total
Newest house number				1(8.3 3%)			1(8.3 3%)		10(83 .33%)	12

Table 26 Rearing farms supplier risks

	Responded	**Yes**	**No**
Are you willing to accept chicks / eggs from a breeder where birds were previously diagnosed as positive for Salmonella enteritidis?	13	38.46	61.54
Arrival bacteriology Salmonella spp.	13	92.31	7.69
Do you put birds from multiple suppliers (Grandparent/ multiplier flock) in the same house?	13	92.31	7.69
Does farm operate on an all in all out production system?	13	92.31	7.69
Do the houses operate on an all in all out production system?	13	100	

Broiler layer farms

Table 27 Broiler layer farms oldest house

	< 5 years	5-10	11-15	16-20	>20	Total
Oldest house (number of houses)			2(10.53)		17(89.47)	19

Table 28 Broiler layer farms newest house

	<1	***1-2***	***>2-3***	***>3-4***	***>4-5***	***>5-10***	***>10-15***	***>15-20***	***> 20***	***Total***
Newest house number				1(5.2 6)			1(5.2 6)		17(8 9.47)	19

Table 29 Broiler layer farm general factors

	Responded	Yes	No	Unsur e
Has the farm been previously infected with SE?	20	90	10	
Are samples taken when chickens arrive at the farm?	15	6.67	93.33	
Do you put birds from multiple suppliers (Grandparent/ multiplier flock) in the same house?	20	100		
Does farm operate on an all in all out production system?	20		100	
Do the houses operate on an all in all out production system?	20	100		
Eggs disinfected at the farm	20	100		
Is egg collection automated?	17		100	
Are nest boxes on top of each other	17		100	
Are samples taken: when chicks arrive at the farm?	15	6.67	86.67	6.67
Are samples taken: at the onset of lay?	20	30	65	5
Are samples taken: at thermal extremes (When it is very hot or very cold)?	15	6.67	86.67	6.67
On set of lay bacteriology Salmonella spp.	7	85.71	14.29	
On set of lay bacteriology Mycobacterium avium complex	7	42.86	57.14	
On set of lay bacteriology Enterococci	7	14.29	85.71	

Broiler farms

Small broiler farms

Table 30 Small broiler farm general risk factors

Factor	Responded	Yes	No	Unsure
Do you or any of your employees have immediate family (people living in the same house) that works on or for another poultry farm?	7	28.57	71.43	
Are the chickens' free range?	7	85.71	14.29	
Do you put birds from multiple suppliers (Grandparent/ multiplier flock) in the same house?	7	14.29	85.71	
Does farm operate on an all in all out production system?	7	28.57	57.14	14.29
Do the houses operate on an all in all out production system?	7	71.43	28.57	
Do you ask what the disease status of the farm that supplies chicks to you is?	7	14.29	57.14	28.57

Large Broiler farms

Factor	Responded	Yes	No
Do you or any of your employees have immediate family (people living in the same house) that works on or for another poultry farm?	40	85	15
Do you put birds from multiple suppliers (Grandparent/ multiplier flock) in the same house?	40	82.5	17.5
Does farm operate on an all in all out production system?	40	82.5	17.5
Do the houses operate on an all in all out production system?	40	100	
Do you ask what the disease status of the farm that supplies chicks to you is?	40	82.5	17.5
Are samples taken:[when chicks arrive at the farm?	34	79.41	20.59
Additional drinker fonts on day one	40	18	82
Arrival bacteriology Salmonella spp.	28	96.43	3.57

Table 31 Large broiler farm visitors

	Min	average	Max	Std Dev.
Litter suppliers	0	0	0	0.00
Veterinarians	0	4.5	11	3.02
Pest control service providers	0	3.6	24	8.57
Disinfectant suppliers	0	18.6	24	8.88
Cleaning team	55	264	330	105.22
Manure and dead bird removals	0	41.9	55	20.97
Advisors	0	4.3	24	8.28
Local vendors	0	0	0	0.00
State employees	0	0.85	1	0.36
Hobby poultry keepers	0	0	0	0.00

In Table 31 the number represents the number of days a person would be on a farm. For examples if 30 people visit on one day they number would be 30 and if 1 person visits for 30 days the number would also be 30.

Abattoirs

Table 32 Abattoir factors

	Responded	Yes	No	Unsure
External certification for Prerequisite programs	5	3	2	
FSSC 22000	5	2	3	
Retail certification	5	4	1	

	Responded	Yes	No	Unsure
Do you or any of your employees have immediate family (people living in the same house) that works on or for another poultry farm?	5	1	3	1
Species slaughtered Layer Chickens	5	1	4	
Is free range and label chickens processed at the establishment?	5	3	2	
Is the health status of the flock in terms of Salmonella provided to the abattoir?	5	3	2	
Are blue lights used at hanging?	5	1	4	
Is a breast comforter used?	5	3	2	
Is there Mechanical (Automatic) evisceration?	5	3	2	
Are the people at evisceration trained to adjust the machine to accommodate for carcass size?	5	3	2	
Is there a pre-scalding wash?	5	1	4	
Does the scalding bath use counter current flow of water?	4	0	3	1
Rehanging of carcasses is done mechanically	4	3		1
All chicken carcasse`s that are dropped on the floor are condemned	4	2	1	1
All chicken pieces that are dropped on the floor are trimmed	4	3		1
Is water recycled through the scalding tanks?	5	4	1	
Application of chlorine following de-feathering	5	2	3	
Application of chlorine dioxide following de-feathering	5	2	3	
Application of chlorine following evisceration	5	4	1	
Application of chlorine dioxide following evisceration	5	1	4	
What type of chilling is used? - Air Chilling	5	4	1	
What type of chilling is used? - Forced air chilling (Blast chilling)	5	2	3	
What type of chilling is used? - Immersion Chilling	5	1	4	
Do you use water sprays to prevent desiccation of carcasses?	4	1	3	
Are the carcasses and water sprays arranged to reduce cross contamination?	5	2	2	1
Is the packaging leak proof?	4	4		

	Responded	Yes	No	Unsure
Is the packaging vacuum-packed?	4		3	1
Is there any decontamination of the outside of the packaging?	4		4	
At final packing does the company know which carcasses or chicken pieces are likely to be contaminated with Salmonella?	4	3	1	
Are the carcasses that are known to be higher risk for containing salmonella treated any different from the rest?	4	1	1	2
During slaughter are samples collected for Salmonella?	5	4	1	
Are samples taken: during transportation of chickens to the slaughter- house?	3	1	2	
Is the water supply ever tested for bacteria?	5	4	1	
During which half of slaughter are samples collected for Salmonella? 1st Half	5	3	2	

Principal component analysis tables

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10	Dim.11
Variance	6.895	2.439	1.183	1.03	0.758	0.678	0.579	0.458	0.268	0.247	0.213
% of var.	45.967	16.263	7.89	6.868	5.052	4.52	3.857	3.052	1.787	1.645	1.423
Cumulative % of var.	45.967	62.229	70.119	76.987	82.04	86.56	90.418	93.47	95.257	96.902	98.325

$Dim.1		
$Dim.1$quanti		
	Correlation	p.value
Testing for faecal coliforms- CP9[SQ2]	0.981557	$2.54887x10^{-38}$
Testing for salmonella after cleaning- CP9[SQ3]	0.981557	$2.54887x10^{-38}$
Producer.L	0.980803	$7.01728x10^{-38}$
The use of organic acids in feed or water - MP1[SQ8]	0.910637	$3.23289X10^{-21}$
SQA1_SQ2	0.86219	$1.10889X10^{-16}$
Number of Casual Staff above mean	0.739595	$2.51743X10^{-10}$
Surface area above the mean	0.70142	$4.91699X10^{-09}$
Full.time.staff.larger	0.495336	0.000162605
Hatchery.more.than.1	0.407212	0.002476479
Positive	0.321988	0.01871267
Newesthouse.15yrs.More	0.274826	0.04641617
Loc23	-0.36682	0.006897888
MP1_SQ9	-0.75991	$4.15837X10^{-11}$

$Dim.2

$Dim.2$quanti		
	correlation	p.value
Locm36	0.7488192	1.14E-10
Abattoir.Loc29	0.7221297	1.04E-09
Loc23	0.7183224	1.40E-09
Newesthouse.15yrs.More	0.575523	6.54E-06
Positive	0.519256	6.78E-05
Full.time.staff.larger	0.2962563	3.12E-02

$Dim.3

$Dim.3$quanti		
	correlation	p.value
Casual.Staff	0.5188376	6.89E-05
Surface.area	0.3666049	6.93E-03
Hatchery.more.than.1	-0.7745403	1.02E-11

$Dim.4

$Dim.4$quanti		
	correlation	p.value
Positive	0.5388892	3.14E-05
Newesthouse.15yrs.More	-0.4746612	3.29E-04
Full.time.staff.larger	-0.4957049	1.61E-04

Water

Rearing farms

Table 33 Rearing farms water bacteriology and source

	Responded	Yes		No	
Water tested for Escherichia coli	13	92.31		7.69	
Water tested for Faecal coliforms	13	92.31		7.69	
Water tested for Salmonella spp.	13	38.46		61.54	
Water tested for Total plate count (TPC/TVC)	13	92.31		7.69	
Water treatment- Filtration	10	10			
Water treatment- Chlorination	10	2		8	
Water treatment- Chlorine dioxide	10	7		3	
	Responded	**WellPoint or Borehole**	**Municipality**	**Dam**	**River**
Source of drinking water for the birds?	10	80	0	0	20

Broiler layer farms

Table 34 Broiler layer farms water bacteriology and source

	Responded	Yes		No	
Is the water supply ever tested for bacteria?	20	100		0	
Water tested for Escherichia coli	20	95		5	
Water tested for Faecal coliforms	20	95		5	
Water tested for Salmonella spp.	20	30		70	
Water tested for Total plate count (TPC/TVC)	20	90		10	
Water treatment- Filtration	16	2			
Water treatment- Chlorination	16	3		13	
Water treatment- Chlorine dioxide	16	13		3	
		WellPoint or Borehole	Municipality	Dam	River
Source of drinking water for the birds?	17	41.18	35.29	5.88	17.65

Broiler farms

Small broiler farms

Table 35 Small broiler farmers water source

	Responded	WellPoint or Borehole	Municipality	River
Where do you source the drinking water for the birds?	7	42.86	42.86	14.29
Water testing	No water testing was reported from any of the respondents			

Large broiler farms

Factor	Responded	WellPoint or Borehole	Dam	Municipality	River
Where do you source the drinking water for the birds?	37	97		3	
		Yes		**No**	
Water tested for Salmonella spp.	39	15.38		84.62	
Water tested for Total plate count (TPC/TVC)	45	86.67		13.33	

Abattoirs

	Responded	Yes	No
Water tested for Escherichia coli	4	4	
Water tested for Faecal coliforms	4	4	
Water tested for Salmonella spp.	4	0	4
Water tested for Enterococci	4	1	3
Water tested for Total plate count (TPC/TVC)	4	4	

Pest control and cleaning

Rearing farms

Table 36 Rearing farms pest control

	Responded	Yes	No
Is there a pest control program?	13	100	
Are houses accessible to wild birds	13	7.69	92.31
Pest seen in last 6 months :Rodents	13	38.46	61.54
Pest seen in last 6 months :Poultry Red mite	13		100
Pest seen in last 6 months :Flies	13	100	

Pest seen in last 6 months :Litter beetle	13	38.46		61.54	
Cleaning bacteriology Escherichia coli	13	30.77		69.23	
Cleaning bacteriology Faecal coliforms	13	30.77		69.23	
Cleaning bacteriology Salmonella spp.	13	38.46		61.54	
Cleaning bacteriology Total plate count (TPC/TVC)	13	61.54		38.46	
	Responded	**Operational / existing staff**	**External company**	**Separate group of staff dedicated to cleaning**	**Other**
Who does the cleaning?	13	7.69	30.77	53.85	7.69

Broiler layer farms

Table 37 Broiler layer pest control and bacteriology done after cleaning

	Responded	Yes	No
Is there a pest control program?	20	100	
Are houses accessible to wild birds	20	90	10
Pest seen in last 6 months :Rodents	20	100	
Pest seen in last 6 months :Poultry Red mite	20	0	100
Pest seen in last 6 months :Flies	20	100	
Pest seen in last 6 months :Litter beetle	20	25	75
Are the cleaning chemicals used SABS approved for efficacy against SE?	20	100	
Are bacteriological samples been taken after cleaning to verify that the cleaning was effective?	20	100	
Cleaning bacteriology Escherichia coli	20		100
Cleaning bacteriology Faecal coliforms	20		100
Cleaning bacteriology Salmonella spp.	20	25	75
Cleaning bacteriology Total plate count (TPC/TVC)	20	90	10

	Responded	Operational / existing staff	External company	Separate group of staff dedicated to cleaning	Other
Who does the cleaning?	13	7.69	30.77	53.85	7.69

Broiler farms

Small broiler farms

	Responded	Yes	No
Is there a pest control program?	7	71.43	28.57
Are houses accessible to wild birds	7	28.57	71.43
Pest seen in last 6 months :Rodents	7	57.14	42.86
Pest seen in last 6 months :Poultry Red mite	7		100
Pest seen in last 6 months :Flies	7	71.43	28.57
Pest seen in last 6 months :Litter beetle	7		100
Cleaning bacteriology Total plate count (TPC/TVC)	20	90	10

	Responded	Operational / existing staff	External company	Separate group of staff dedicated to cleaning	Other
Who does the cleaning?	7	100			

Large broiler farms

	Responded	Yes	No
Is there a pest control program?	39	100	
Are houses access able to wild birds?	40	17.5	82.5
Pest seen in last 6 months :Rodents	41	31.71	68.29
Pest seen in last 6 months :Poultry Red mite	41		100
Pest seen in last 6 months :Flies	41	97.56	2.44
Pest seen in last 6 months :Litter beetle	41	80.49	19.51
Cleaning bacteriology Escherichia coli	40	15	85
Cleaning bacteriology Faecal coliforms	40	15	85

	Responded	Yes		No	
Cleaning bacteriology Salmonella spp.	40	15		85	
Cleaning bacteriology Campylobacter spp.	41	65.85		34.15	
Cleaning bacteriology Total plate count (TPC/TVC)	41	65.85		34.15	
Cleaning bacteriology Total plate count (TPC/TVC)	20	90		10	
	Responded	**Operational / existing staff**	**External company**	**Separate group of staff dedicated to cleaning**	**Other**
Who does the cleaning?			32.5	67.5	

Abattoirs

	Responded	Yes		No	
Bacteriological samples been taken after cleaning to verify that the cleaning was effective?	5	4		1	
Cleaning bacteriology Escherichia coli	5	4		1	
Cleaning bacteriology Faecal coliforms	5	3		2	
Cleaning bacteriology Salmonella spp.	5	1		4	
Cleaning bacteriology Enterococci	5	1		4	
Cleaning bacteriology Total plate count (TPC/TVC)	5	4		1	
	Responded	**Operational / existing staff**	**External company**	**Separate group of staff dedicated to cleaning**	**Other**
Who does the cleaning?	5	1	3	67.5	

Medication and vaccination

Rearing farms

Table 38 Rearing farms medication and vaccination

Factor	Responded	Yes	No	Unsure
Has antibiotics used in the last 12 months?	13	92.31		7.69

Factor	Responded	Yes	No	Unsure
Has coccidiosis preventive medicines used in the last 12 months?	13	84.62	7.69	7.69
Other medication used in the last 12 months?	13	7.69	84.62	7.69
Probiotics used in the poultry feed	13	53.85	38.46	7.69
Do you use organic acids in the feed or water?	13	38.46	53.85	7.69
Hatchery Vaccination :Marek's Disease	13	92.31	7.69	
Hatchery Vaccination :Newcastle Disease	13	92.31	7.69	
Hatchery Vaccination :Infectious Bronchitis	13	92.31	7.69	
Farm vaccination :Mycoplasma gallisepticum	13	53.85	46.15	
Farm vaccination: Salmonella	13	100		
De-wormer used	13	Levamisole or Abermectin		
Antibiotic actives given in the last 12 months	13	Virginiamycin		

Broiler layer farms

Table 39 Broiler layer farms medication and vaccination

	Responded	Yes	No	Unsure
Antibiotics used in the last 12 months	20	85	10	5
Coccidiosis preventive medicines used in the last 12 months	20	0	95	5
De-wormer used in the last 12 months	20	100	0	0
Probiotics in poultry Feed	20	65	30	5
Organic acids in the feed or water	20	90	5	5
Farm vaccination :Mycoplasma gallisepticum	20	0	100	0
Farm vaccination :Newcastle Disease	20	100	0	0
Farm vaccination :Infectious Bronchitis	20	100	0	0
Farm vaccination :Infectious Bursal Disease (Gumboro)	20	5	95	0
Farm vaccination :Infectious Coryza	20	5	95	0
Farm vaccination :Salmonella enteritidis	20	30	70	0
Farm vaccination :Mycoplasma gallisepticum	20	5	95	0
Farm vaccination :Fowl Pox	20	5	95	0
De-wormer actives used	Abermectin, Fenbendazole			
Antibiotic used	Chlortetracycline; Kanamycin monosulphate; Virginiamycin			

Broiler farms

Small broiler farms

	Responded	Yes	No	
Anti-parasitisides (mites, worms) used in the last 12 months?	7	14.29	85.71	
Antibiotics used in the last 12 months?	7	28.57	71.43	
Coccidiosis preventive medicines used in the last 12 months?	7	28.57	71.43	
Medication for external parasites used in the last 12 months?	7	14.29	85.71	
Are smaller birds culled?	6	16.67	83.33	
Do you use probiotics in the poultry or cleaning agents?	7	42.86	57.14	
Do you use organic acids in the feed or water?	7	14.29	85.71	
Anti coccidials	Monensin and nicarbazin			

Large broiler farms

	Responded	Yes	No
antibiotics used in the last 12 months?	40	82.5	17.5
coccidiosis preventive medicines used in the last 12 months?	40	82.5	17.5
medication for external parasites used in the last 12 months?	40	67.5	32.5
Probiotics in the poultry feed or water?	39	84.62	15.38
Organic acids in the feed or water?	39	15.38	84.62
Hatchery Vaccination :Marek's Disease	40	15	85
Hatchery Vaccination :Newcastle Disease	46	97.83	2.17
Hatchery Vaccination :Infectious Bronchitis	46	97.83	2.17
Hatchery Vaccination	46	74%	26%
Farm vaccination :Infectious Bronchitis	45	84.44	15.56
Antibiotics	Doxycycline; Enrofloxacin, Trimethoprim Sulfadiazine and Kanamycin		
Anticoccidials	Nicarbazin and Silinomycin Sodium		

Appendix C. Questionnaire

Poultry risk survey 2017-2018

Welcome to the poultry risk survey

[IC1]
I hereby give permission for collection of data and thereby agree to take part in the research project entitled: "A risk assessment of Salmonella enteritidis in the broiler poultry of the Western Cape, South Africa"
My data may be used for the study as long as my organisations' and personal details are kept confidential and not revealed in any publications.

[Contact]Please, provide contact details for your self, in case we need some clarification about some of the questions you have answered?

[EC1]What kind of operation is this?

[EC3]What external certifications if any does the establishment have?

[EC4]Please select the location on the map

[EC9]Please answer yes or no to the following qestions

	Yes	Uncertain	No
Are vehicles disinfected at entrance or exit from the establishment?	○	○	○
Is there a designated parking area?	○	○	○
Do staff cross the parking area during the working day? (is the parking area within the production area?)	○	○	○
Are there signs that prohibit un-authorised entrance to the premises?	○	○	○
Are there any other animals or birds other than chickens on the premises?	○	○	○
Do you or any other employees attend poultry or bird shows?	○	○	○
Do you or any other employees own any poultry or birds as pets?	○	○	○
Do you or any of your employees live on a poultry farm?	○	○	○
Do you or any of your employees have immediate family (people living in the same house) that works on or for another poultry farm?	○	○	○
Are there multiple flocks / Houses on the farm?	○	○	○

[EC8]
Please provide the capacity per year (in Metric tons or number) if applicable.

[EC8]Please provide the following statistics:

[EC2]How old is the establishment?

[MH3]How old is the newest house on the farm?

[MH1]How old is the oldest house on the farm?

- < 5 Years
- 5-10 Years
- 10-15 Years
- 15-20 Years
- >20 Years

[EC15]Please indicated the number of the following animals if applicable.

- Sheep or goats
- Dogs or cats
- Horses / donkeys / mules
- Ducks or geese
- Game

[EC11]What disinfectant is used for disinfection of vehicles?

[DisAppl]How is the vehicle disinfectant applied?

- Other

[MH4]Please answer yes or no to the following questions:

[illegible]

	Yes	Uncertain	No
Do the houses share conveyor belts or augers?	○	○	○
Does each house have its own set of equipment?	○	○	○
Does each house have its own workers for daily tasks?	○	○	○
Is there a place at each house for the workers to wash boots and hands before entering the house?	○	○	○
Are there foot baths for every house?	○	○	○
Is equipment cleaned before use in another house?	○	○	○

[FC1]Please provide the amounts for the following:

Only numbers may be entered in these fields.

Please write your answer(s) here:

How many hatcheries supplied the farm in the last year?

How many transport companies have been used in the last year to transport chickens or feed to and from the farm?

How many breeder farms supply the farm?

How many birds are on the farm when the farm is in full production?

How many kilometres away is the nearest poultry farm?

[FC2]Please supply the names and contact details of the hatcheries?

Please write your answer here:

[FC4]Please provide the names and contact details for the transport companies?

Please write your answer here:

[FC5]Please provide the names and contact details of the poultry farms that supplied chickens to your farm?

Please write your answer here:

[FC9]Please answer yes or no to the following questions

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Do you put birds from multiple suppliers (grandparent, multiplier flock) in the same house?	○	○	○
Is the grass kept short around the houses?	○	○	○
Is there a concrete border around the house?	○	○	○
Does farm operate on an all in all out production system?	○	○	○
Do the houses operate on an all in all out production system?	○	○	○
Are all the birds on your farm only one age at a given time?	○	○	○
Are all birds for a house received at the same time?	○	○	○
Are all birds shipped from a house at the same time?	○	○	○
Is feed withdrawn before the birds are transported for slaughter?	○	○	○
Are the chickens' free range?	○	○	○
Are there layers with different ages on the farm?	○	○	○
Are the houses that are being partially depopulated always caught before those that are fully depopulated on the same day?	○	○	○
Are there shower facilities which are used by workers on the farm?	○	○	○
Do the shower facilities have hot and cold water?	○	○	○
Do the shower facilities have soap?	○	○	○

[FC31]How many hours before catching is feed withdrawn?

Only answer this question if the following conditions are met:

[illegible]

Only numbers may be entered in this field.

Please write your answer here:

[FC21]What kind of bedding is used inside the houses if any?

Check all that apply

Please choose **all** that apply:

- ☐ [illegible]
- ☐ Sand
- ☐ [illegible]
- ☐ Paper
- ☐ [illegible]
- ☐ [illegible]
- ☐ Other:

[FC22]Have you ever been informed by the hatchery that the chicks could come from a farm potentially infected with Salmonella?

Please write your answer here:

[FC24]How often have you used a veterinarian in the last year?

Check all that apply

Please choose all that apply:

- Once a year
- Quarterly
- Monthly
- Weekly
- Daily
- Every production cycle
- Other:

[FC34]What are the main reasons you use the veterinarian?

Please write your answer here:

[FC28]Please describe how manure is managed? (How often is it removed from houses? where is it stored? and what does it get used for? How is the area/ house disinfected after the manure is removed?)

Please write your answer here:

[FC29]How many meters is manure stored from the poultry house?

Only numbers may be entered in this field.

Please write your answer here:

[FC30]What is the longest amount of time that manure is stockpiled on the farm?

Please write your answer here:

Please specify the unit of measurement. For example is it years, months, weeks or days etc.

[FC32][illegible]

Please choose the appropriate response for each item:

	Strongly disagree 1	2	3	4	Strongly agree 5
Manure is stock piled on my farm and it is not a risk to my chickens	○	○	○	○	○
Workers shower daily before entering the poultry house	○	○	○	○	○
The hatchery that supplies my farm will inform me if Salmonella enteritidis was detected from a sample above or below the hatching crate my chicks come from	○	○	○	○	○

[MP1]Please indicate yes or no to the following questions

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Is moulting practiced?	○	○	○
Is moulting done by withholding feed and water?	○	○	○
Has anti-parasiticides (mites, worms) been used in the last 12 months?	○	○	○
Has antibiotics been used in the last 12 months?	○	○	○
Has coccidiosis preventive medicines been used in the last 12 months?	○	○	○
Has dewormer been used in the last 12 months?	○	○	○
Has medication for external parasites been used in the last 12 months?	○	○	○
Has other medication been used in the last 12 months?	○	○	○
Are smaller birds culled?	○	○	○
Do you use probiotics in the poultry or cleaning agents?	○	○	○
Do you use organic acids in the feed or water?	○	○	○
Are there any diseases or pests on the farm?	○	○	○
Do the birds receive any additional vaccines on the farm?	○	○	○
Is there a pest control program?	○	○	○
Are workers responsible to report the presence of pests?	○	○	○

[MP3]If moulting is not done by withholding feed or water, how is moulting done?

Only answer this question if the following conditions are met:
Answer was 'No' at question '10 [MP1]' (Please indicate yes or no to the following questions (Is moulting done by withholding feed and water?))

Please write your answer here:

[MP18]Describe how smaller birds are culled?

[MP5]
If you have this information, for each of the following that were used, please specify the name, active ingredients, the route of administration (feed, water, injection), the dosage and the duration of application.

	Trade names	Active ingredients	Route of application (Water feed, injection etc..)	Dose	Duration of treatment
anti-parasitaries					
antibiotic used					
dewormers					
medication for external parasites					
coccidiosis preventive medicines					
supplements or additives (Organic acids, probiotics, clay binders etc..)					

[FH2]Please list the diseases known to be on the farm?

[FH4]What were the chicks vaccinated with at the hatchery?

- [ ] Marek's Disease
- [ ] New Castle Disease
- [ ] Infectious Bronchitis
- [ ] Other:

[FH6]What vaccines are used on the farm and how often are they applied?

- [ ] Marek's Disease
- [ ] New Castle Disease
- [ ] Infectious Bronchitis
- [ ] Infectious Bursal Disease (Gumboro)
- [ ] Infectious Coryza
- [ ] Salmonella enteritidis
- [ ] Avian Influenza
- [ ] Mycoplasma gallisepticum
- [ ] Fowl Pox

Other:

[FH9]Which of the following pests were seeen on the farm during the last 6 months?

- [ ] Rodents
- [ ] Poultry Red mite
- [ ] Other Mites
- [ ] Lice
- [ ] Flies
- [ ] Other flying insects
- [ ] Litter beetle
- [ ] Other Crawling insects
- [ ] Other:

[FH91]Please answer yes or no to the following questions:

	Yes	Uncertain	No
Is there a fly control program?	O	O	O
Is there a beetle control program?	O	O	O
Has the farm been previously infected with SE?	O	O	O
Has the farm been infected with poultry red mite (Dermanyssus gallinae)	O	O	O
Has the farm been infected with campylobacter spp.?	O	O	O
Is the minimal ventilation measured for controlled houses?	O	O	O
Are any of the houses accessible to wild birds?	O	O	O
Is mash feed given instead of granular feed (heat treated) on day 1?	O	O	O

[E1]How would you describe the minimum amount of fresh air needed for the young chicks? and do you measure it?

Please write your answer here:

[MPwater]What kind of watering equipment is present on day 1?

Please write your answer here:

[H1]How many farms supply the hatchery?

Only numbers may be entered in this field.
Please write your answer here:

[H2]How many farms does the hatchery supply?

Only numbers may be entered in this field.
Please write your answer here:

[H3]Please answer yes or no to the following questions

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Does the hatchery ensure that the known SE negative farms only receives chicks from a SE negative farm?	○	○	○
Does the hatchery ensure that disease-negative eggs are hatched in different incubators or at different times from eggs from farms with unknown status farms and known positive farms	○	○	○
Are the shells disinfected before being put into the incubator?	○	○	○
Based on the supplier of eggs, is there a disease classification for the day old chicks that are sent out?	○	○	○
Are the eggs from known salmonella infected farms transported separately from disease free farms?	○	○	○
Are the chicks from salmonella infected farms handled separately from non infected farms?	○	○	○
Are egg trays shared between farms?	○	○	○

[H7]How does the disease classification of chicks work?

Only answer this question if the following conditions are met:
Answer was 'Yes' or 'Uncertain' at question '13 [H3]' (Please answer yes or no to the following questions. (Based on the supplier of eggs, is there a disease classification for the day old chicks that are sent out?))

Please write your answer here:

[H8]What are the chick routinely vaccinated with at the hatchery?

Only answer this question if the following conditions are met:
Answer was 'Yes' or 'Uncertain' at question '13 [H3]' (Please answer yes or no to the following questions.)

Please write your answer here:

[H9]What additional vaccines are available on request?

Please write your answer here:

[H11]When is a farm classified as salmonella free?

Please write your answer here:

[H10]When is a farm classified as campylobacter free?

Please write your answer here:

[L14]What products are used to disinfect the shells?

Please write your answer here:

[H16]Please describe that process of cleaning chick handling crates? Please include the frequency, method as well as the chemicals used for cleaning and disinfection.

Please write your answer here:

[H17]Describe that process of cleaning egg trays? Please include the frequency, method as well as the chemicals used for cleaning and disinfection.

Please write your answer here:

[L1]Please answer yes or no to the following questions

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Are there nest boxes?	○	○	○
Are the eggs disinfected at the farm?	○	○	○
Are the eggs disinfected at the hatchery?	○	○	○
Are the nest boxes on top of each other?	○	○	○
Is egg collection automated?	○	○	○

[L2]What types of bedding are used in nest boxes?

Only answer this question if the following conditions are met:
Answer was 'Uncertain' or 'Yes' at question '32 [L1]' (Please answer yes or no to the following questions (Are there nest boxes?))

Check all that apply
Please choose all that apply:

- ☐ Straw
- ☐ Sand
- ☐ Soft Wood shavings
- ☐ Paper
- ☐ Seed hulls
- ☐ Hard wood shavings
- ☐ Other:

[L4]How many times per day are eggs collected?

Only answer this question if the following conditions are met:
Answer was 'Uncertain' or 'No' at question '32 [L1]' (Please answer yes or no to the following questions (Is egg collection automated?))

Only numbers may be entered in this field.
Please write your answer here:

[L5]How are the chicks moved from the rearing site to the egg producing farm?

Please write your answer here:

Own transport, customer transport, specialist transport company. Open truck, closed truck, at night, early morning. Truck cleaned before loading, the truck is used for one farm only?

[L7]How is the outside area cleaned?

Please write your answer here:

[L8]What is the frequency of cleaning the nest boxes?

Please write your answer here:

[L9]What chemicals are used to disinfect the eggs at the farm?

Only answer this question if the following conditions are met:
Answer was 'Yes' or 'Uncertain' at question '32 [L1]' (Please answer yes or no to the following questions (Are the eggs disinfected at the farm?))

Please write your answer here:

[L10]How are the eggs disinfected?

Only answer this question if the following conditions are met:
Answer was 'Yes' or 'Uncertain' at question '32 [L1]' (Please answer yes or no to the following questions (Are the eggs disinfected at the hatchery?))

Please write your answer here:

[L11]What is the longest period from laying until the eggs are disinfected?

Please write your answer here:

[L12]How soon after collection are eggs disinfected?

Please write your answer here:

[LDisEggsHatch]

Please write your answer here:

[SQA1]Please answer yes or no to the following questions:

	Yes	Uncertain	No
Do you see what the disease status of the farm that supplies chicks to you is?	○	○	○
Are you willing to accept chicks / eggs from a breeder where birds were previously diagnosed as positive for Salmonella enteritidis?	○	○	○
Are you willing to accept chicks/eggs from a breeder where birds were previously diagnosed as positive for Campylobacter spp?	○	○	○
Does the hatchery ensure that the farm only receives chicks from a SE negative farm and that they were hatched in different incubators or at different times from unknown status farms and known previously diagnosed as positive farms?	○	○	○
Does your feed supplier have GMP's in place and are they certified by an external company?	○	○	○
Do you ever sample and test the feed for salmonella?	○	○	○
Does the feed truck park near the change room?	○	○	○
Does the feed truck get disinfected on entry and exit from your farm (high pressure wash / over head spray)?	○	○	○
Does the supplier stop at other farms along the route to deliver feed to your farm?	○	○	○

[SQA8]How often does the feed supplier visit the farm on a monthly basis other than to deliver feed?

Only numbers may be entered in this field.

Please write your answer here:

[SQA10]How often is feed delivered to the farm in one month?

Only numbers may be entered in this field.

Please write your answer here:

[S1]Are samples taken:

Please choose the appropriate response for each item:

	Yes	Uncertain	No
when chicks arrive at the farm?	○	○	○
at the onset of lay?	○	○	○
at thermal extremes (When it is very hot or very cold)?	○	○	○
during transportation of chickens to the slaughter-house?	○	○	○
from each hatching basket at the hatchery?	○	○	○
Does the hatchery warn the farmer if there was a positive sample above or below the basket where the chicks hatched?	○	○	○
Is the water supply ever tested for bacteria?	○	○	○

[S9]During which half of slaughter are samples collected for Salmonella?

Check all that apply

Please choose **all** that apply:

- ☐ 1st half
- ☐ 2nd half
- ☐ [illegible]
- ☐ Other:

[S11]Select the bacteria that the water is tested for and enter how often the water is tested for that specific bacteria in one year?

Please choose all that apply and provide a comment:

- ☐ Escherichia coli
- ☐ Faecal coliforms
- ☐ Salmonella spp
- ☐ [illegible]
- ☐ Clostridium
- ☐ Campylobacter spp
- ☐ Enterococcus spp
- ☐ Total plate count (TPC/TVC)

[S12]Select the bacteria test that is done on the samples taken on arrival at the farm after chickens are moved and indicate how often these samples are tested in one year?

[S13]For bacterial samples taken on arrival at the farm. Is it possible to reference the farm where the chickens originated from to the samples collected?

[S14]Select the bacterial test that is done on the samples taken at the onset of lay and indicate how often the sample is tested in one year?

[S15]Is it possible to reference the (parent or grandparent) farm of origin to the samples collected at the onset of lay

[S16]Select the bacterial test that is done on the samples taken at thermal extremes and indicate how often the sample is tested in one year?

[S17]For samples taken at thermal extremes, is it possible to reference the house of origin to the samples collected?

[S18]Select the bacterial test that is done on the samples taken during transportation of chickens to the slaughter- house and indicate how often the sample is tested in one year?

[S19]For samples taken during (or immediately after) transportation of chickens to the slaughter- house is it possible to reference the farm of origin to the samples collected?

[S22]Select the bacterial test that is done on the samples taken from each hatching basket and indicate how often the sample is tested in one month?

[S23]Is it possible to reference the farm of origin to the samples collected from each hatching basket?

[CP1]Are the cleaning chemicals used SABS approved for efficacy against SE?

[CP2]How often are the houses cleaned?

[CP3]How are the poultry houses cleaned and disinfected? Please include the names of the chemicals used as well as the concentration used and contact time.

[CP4]Who does the main cleaning?

[CP5]Are bacteriological samples been taken after cleaning to verify that the cleaning was effective?

[CP7]How many samples are taken after cleaning one house?

[CP8]What kind of samples are taken after cleaning?

[CP9]What bacteria are tested for after cleaning and how often are tests done in one year?

Comment only when you choose an answer.
Please choose all that apply and provide a comment:

- ☐ Escherichia coli
- ☐ Fecal coliforms
- ☐ Salmonella spp.
- ☐ Mycobacterium avium complex
- ☐ Clostridium
- ☐ Campylobacter spp.
- ☐ Enterococci
- ☐ Total plate count (TPC/TVC)

Other:

[GB1]Please answer yes or no to the following questions.

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Is there a worker training program?	○	○	○
Is there a cleaning program?	○	○	○
Are there dirt roads within 1km of the Establishment?	○	○	○
Is there a dust control program?	○	○	○
Do you treat the water used (i.e. filtration, ozonation, chlorination, microstrainers)?	○	○	○
Do you actively check if any of your staff members have poultry?	○	○	○
Do you do any community poultry vaccination?	○	○	○
Is worker hygiene part of the worker training program?	○	○	○

[GB11]What is the shortest distance in meters from a building to a dirt road if applicable?

Only numbers may be entered in this field.
Please write your answer here:

Please use meters (m)

[BG5]How is dust controlled?

Please write your answer here:

[GB6]Where do you source the drinking water for the birds?

Only answer this question if the following conditions are met:

Choose one of the following answers
Please choose **only one** of the following:

- ○ Well / borehole
- ○ Municipal system
- ○ Dam
- ○ River
- ○ Other

[GB8]Please describe the water treatment?

Only answer this question if the following conditions are met:
Answer was 'Yes' or 'Uncertain' at question '[GB1]' (Please answer yes or no to the following questions. (Do you treat the water used (i.e. filtration, ozonation, chlorination, microstrainers)?))

Please write your answer here:

[GB12]What vaccination do you do in the community?

Only answer this question if the following conditions are met:
Answer was 'Yes' or 'Uncertain' at question '[GB1]' (Please answer yes or no to the following questions. (Do you do any community poultry vaccination?))

Please write your answer here:

[GB14]How often are dead birds removed from the houses?

Only answer this question if the following conditions are met:

Please write your answer here:

[GB13]How do you manage the dead birds?

Only answer this question if the following conditions are met:

Choose one of the following answers
Please choose **only one** of the following:

- ○ Incineration
- ○ Burial
- ○ Manure pit
- ○ Rendering
- ○ Composting
- ○ Pig farms
- ○ Other

[OB15]Please describe how manure is managed?

[WT2]What is included in the worker training program?

[ECOther]
Other than feed suppliers (Other than deliveries), litter suppliers, veterinarians, pest control service providers, disinfectant suppliers, cleaning team, manure and dead bird removals, advisors, local vendors, state employees and Hobby poultry keepers what people or service providers visit your establishment?

[FS1]How often do the following suppliers come to the property?

	Once a year	Quarterly	Monthly	Weekly	Every production cycle	Multiple times per production cycle	Never
Litter suppliers	○	○	○	○	○	○	○
Veterinarians	○	○	○	○	○	○	○
Pest control service providers	○	○	○	○	○	○	○
Disinfectant suppliers	○	○	○	○	○	○	○
Cleaning team	○	○	○	○	○	○	○
Manure and dead bird removals	○	○	○	○	○	○	○
Advisors	○	○	○	○	○	○	○
Local vendors	○	○	○	○	○	○	○
State employees	○	○	○	○	○	○	○
Hobby poultry keepers	○	○	○	○	○	○	○
{ECOther}	○	○	○	○	○	○	○

[FSPeopleNum]For the following suppliers how many people were there on the last visit?

Feed suppliers (Other than deliveries)	
Litter suppliers	
Veterinarians	
Pest control service providers	
Disinfectant suppliers	
Cleaning team	
Manure and dead bird removals	
Advisors	
Local vendors	
State employees	
Hobby poultry keepers	
{ECOther}	

[FSAccessPol]Please describe the level of access they get on the site?

	Office	Parking	General Grounds	Fenced area around the houses	Feed bins	Empty dirty house	Within the house with birds early production	Within the house with birds late production	Empty clean house	No at all on the farm
Feed suppliers (Other than deliveries)	○	○	○	○	○	○	○	○	○	○
Litter suppliers	○	○	○	○	○	○	○	○	○	○
Veterinarians	○	○	○	○	○	○	○	○	○	○
Pest control service providers	○	○	○	○	○	○	○	○	○	○
Disinfectant suppliers	○	○	○	○	○	○	○	○	○	○
Cleaning team	○	○	○	○	○	○	○	○	○	○
Manure and dead bird removals	○	○	○	○	○	○	○	○	○	○
Advisors	○	○	○	○	○	○	○	○	○	○
Local vendors	○	○	○	○	○	○	○	○	○	○
State employees	○	○	○	○	○	○	○	○	○	○
Hobby poultry keepers	○	○	○	○	○	○	○	○	○	○
{ECOther}	○	○	○	○	○	○	○	○	○	○

[FSPre]If applicable please describe the precautions taken for the level of access granted for each supplier if they are treated differently?

Please write your answer here:

[VisitPPE]Do you supply the clothing for the following suppliers? (Excluding boots/ shoes)

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Feed suppliers (Other than deliveries)	○	○	○
Litter suppliers	○	○	○
Veterinarians	○	○	○
Pest control service providers	○	○	○
Disinfectant suppliers	○	○	○
Cleaning team	○	○	○
Manure and dead bird removals	○	○	○
Advisors	○	○	○
Local vendors	○	○	○
State employees	○	○	○
Hobby poultry keepers	○	○	○
(6)Other)	○	○	○

[VisitShoes]Do you supply shoes or boots?

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Feed suppliers (Other than deliveries)	○	○	○
Litter suppliers	○	○	○
Veterinarians	○	○	○
Pest control service providers	○	○	○
Disinfectant suppliers	○	○	○
Cleaning team	○	○	○
Manure and dead bird removals	○	○	○
Advisors	○	○	○
Local vendors	○	○	○
State employees	○	○	○
Hobby poultry keepers	○	○	○
(6)Other)	○	○	○

[VisitContact]Please provide the name of company/s and contact details of contact person / people for the following?

[FootBath]Are there disinfection baths for shoes before entering each house if boots and / or shoes are not provided?

Only answer this question if the following conditions are met:
[illegible]

Please choose **only one** of the following:

- ○ Yes
- ○ No

[A1]How many farms supply the abattoir?

Only answer this question if the following conditions are met:
Answer was at question '1 [EC1] (What kind of operation is this?)

Only numbers may be entered in this field.
Please write your answer here:

[A2]How many abattoirs supply the cutting plant?

Only answer this question if the following conditions are met:
[illegible]

Only numbers may be entered in this field.
Please write your answer here:

[A10]Please select the species that are slaughtered or cut?

Check all that apply
Please choose **all** that apply:

- ☐ Broiler Chicken
- ☐ Layer Chickens
- ☐ Ducks
- ☐ Rabbits
- ☐ Geese
- ☐ Cattle
- ☐ Sheep
- ☐ Goats
- ☐ Pig
- ☐ Game
- ☐ Other:

[A4]Answer yes or no to the following questions

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Is imported chicken also used in the cutting plant?	○	○	○
Is free range and [illegible] chickens processed at the establishment?	○	○	○
Does the establishment attribute a reference number to a delivery of chicken received?	○	○	○
Does the establishment attribute a reference number to the flock?	○	○	○
Is the health status of the flock in terms of Salmonella provided to the abattoir?	○	○	○
Is the health status of the flock in terms of Campylobacter provided to the abattoir?	○	○	○
Is the light dimmed at receiving and hanging of birds?	○	○	○
Are blue lights used at hanging?	○	○	○
Is there an ante-mortem inspection?	○	○	○
Is a [illegible] used?	○	○	○
Is there Mechanical (Automatic) evisceration?	○	○	○
Are the people at evisceration trained to adjust the machine to accommodate for carcass size?	○	○	○

[A6]
Please include the chemicals used as well as the frequency and method of cleaning for the following:

	chemicals used	frequency of cleaning	method of cleaning
trucks that deliver birds to the abattoir			
the crates that are used to transport birds to the abattoir			
scalding tanks			
de-feathering equipment			

[A7]
How are chicken carcases rinsed?

Choose one of the following answers
Please choose **only one** of the following:

- ○ [illegible]
- ○ [illegible]
- ○ [illegible]

[A13]Please provide a description of the process including all control points and critical control points. Any operational prerequisite programs need to be included namely chillers freezer and any divergence of products because of farm health status need to be included.

Please write your answer here:

[A5]How many people are at evisceration?

Only numbers may be entered in this field.
Please write your answer here:

[A17]What is the policy regarding the withdrawal of feed on the broiler farms?

Please write your answer here:

[A16]How many hours before slaughter should feed be withdrawn if applicable?

Only numbers may be entered in this field.
Please write your answer here:

[A18]If birds are from a flock previously positive/ positive for Salmonella spp. how are they handled?

Please write your answer here:

[A19]If birds are from a flock positive for Campylobacter spp. how are they handled? Please mention, order of slaughter as well as further processing if applicable.

Please write your answer here:

[A20]What is done with unhealthy chickens if detected at antemortem?

[A21]In terms of communication what is the procedure if more sick or dead birds are detected at ante-mortem than what is expected by the abattoir?

[A22]What is the expected number of dead or sick birds at ante-mortem that will result in action being taken?

[A26]What is the shortest amount of time in minutes it takes from the throat cut to the bird entering the scalding tank?

Only numbers may be entered in this field.

[A40]Please answer yes or no to the following questions:

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Is there a pre-scalding wash?	○	○	○
Does the scalding bath use counter current flow of water?	○	○	○
Are chemicals added to the scalding bath?	○	○	○
Is the temperature of the scalding baths raised during breaks?	○	○	○

[A27]

Please rate the following statements from 1 to 5. With 1 being that you do not agree and 5 being that you agree

Please choose the appropriate response for each item:

	1	2	3	4	5
Contamination of carcasses or meat pieces at this facility is reduced by washing with abundant potable water	○	○	○	○	○
Carcasses /pieces are trimmed to remove contamination	○	○	○	○	○
Carcasses /pieces are disposed of when there is extensive faecal contamination	○	○	○	○	○
Carcasses /pieces are reprocessed when there is extensive faecal contamination	○	○	○	○	○
Chemical decontaminants are used to decontaminate carcasses / pieces	○	○	○	○	○
Physical methods are used decontaminated carcasses / pieces	○	○	○	○	○
Rehanging of carcasses is done mechanically	○	○	○	○	○
All chicken carcasses that are dropped on the floor are condemned	○	○	○	○	○
All chicken pieces that are dropped on the floor are condemned	○	○	○	○	○
All chicken carcasses that are dropped on the floor are trimmed and re-washed	○	○	○	○	○
All chicken pieces that are dropped on the floor are trimmed	○	○	○	○	○
All chickens carcasses / pieces that are dropped on the floor are re-washed	○	○	○	○	○

[A43]What chemicals are used in the scalding bath?

[A44]Describe how the concentration of the chemicals is maintained.

[A46]Provide the scalding bath temperature in degrees C.

[A48]What temperature (in degrees C) is reached during breaks?

[A49]How often are the scolding tanks emptied and cleaned? (Number of chickens and time)

[A50]Please answer yes or no to the following questions:

	Yes	Uncertain	No
Are the scalding tanks cleaned daily?	○	○	○
Is water recycled through the scalding tanks?	○	○	○

[A52]Please describe the hygiene measures that are applied to water if it is recycled?

[A54]How is de-feathering done?

[A55]How often is the de-feathering equipment inspected?

[A51]How is feather build-up on equipment prevented?

[A57]How often are the plucker fingers inspected?

[A58]How often are the plucker fingers replaced?

[A56]Is there continues rinsing of de-feathering equipment during operation?

[A59]If there is an application of a chemical (For example ozone, chlorinated water, trisodium phosphate etc..) to the carcase following de-feathering please select the chemical or add the chemical which is applied?

[Aconse]What concentration of the chemical is used?

[A60]What method of application and concentration of the chemical is used?

[A61]Are heads pulled downwards during head pulling?

[A62]If there is an application of a chemical (For example ozone, chlorinated water, trisodium phosphate etc..) to the carcase following evisceration please select the chemical or add the chemical which is applied?

Check all that apply

Please choose all that apply:

- [ ] Acidified sodium Chlorite (ASC)
- [ ] Tri sodium Phosphate (TSP)
- [ ] Chlorine
- [ ] Ozone
- [ ] chlorine gas
- [ ] sodium hypochlorite
- [ ] calcium hypochlorite tablets
- [ ] electrolytically generated hypochlorous acid
- [ ] Citric acid
- [ ] Lactic acid
- [ ] Peracetic acid
- [ ] Peroxy acids
- [ ] hydrogen peroxide
- [ ] chlorine dioxide
- [ ] Other:

[A63]What method of application and concentration of the chemical is used?

Please write your answer here:

[A64]How many times is the carcass washed?

Only numbers may be entered in this field.

Please write your answer here:

[A65]What type of chilling is used?

Check all that apply

Please choose all that apply:

- [ ] Air Chilling
- [ ] Forced air chilling (Blast chilling)
- [ ] Immersion Chilling
- [ ] Other:

[A66]Please answer yes or no to the following questions:

Only answer this question if the following conditions are met:

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Do you use water sprays to prevent desiccation of carcasses?	○	○	○
Are the carcasses and water sprays arranged to reduce cross contamination?	○	○	○
Are there any chemicals contained in the carcass sprays?	○	○	○

[A69]Which of the following, if any, processing aids are used in the water?

Check all that apply

Please choose all that apply:

- [ ] Acidified sodium Chlorite (ASC)
- [ ] Tri sodium Phosphate (TSP)
- [ ] Chlorine
- [ ] Ozone
- [ ] Chlorine gas
- [ ] Sodium hypochlorite
- [ ] Calcium hypochlorite tablets
- [ ] Electrolytically generated hypochlorous acid
- [ ] Citric acid
- [ ] Lactic acid
- [ ] Peracetic acid
- [ ] Peroxy acids
- [ ] Hydrogen peroxide
- [ ] Chlorine dioxide

[A70]Please give the concentration for each chemical used?

[A71]Please answer yes or no to the following questions:

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Is the water flow counter current?	○	○	○
Is the water agitated?	○	○	○
Is there a post chilling application of any chemicals?	○	○	○

[A72]What method of application is used for the post chilling application of chemicals?

Only answer this question if the following conditions are met:

Choose one of the following answers

Please choose only one of the following:

- ○ Spray
- ○ Immersion

[A73]Please select the chemicals used post chilling?

Check all that apply

Please choose all that apply:

- [ ] Acidified sodium Chlorite (ASC)
- [ ] Tri-sodium Phosphate (TSP)
- [ ] Chlorine
- [ ] Ozone
- [ ] Chlorine gas
- [ ] Sodium hypochlorite
- [ ] Calcium hypochlorite tablets
- [ ] Electrolytically generated hypochlorous acid
- [ ] Citric acid
- [ ] Lactic acid
- [ ] Peracetic acid
- [ ] Peroxy acids
- [ ] Hydrogen peroxide
- [ ] Chlorine dioxide
- [ ] Other:

[A74]Indicate the PH of the chemicals added after chilling

[A75]Indicate the concentration of the chemicals added after chilling

[A76]What temperature in degrees Celsius is the portioning area?

Only numbers may be entered in this field.

Please write your answer here:

[A77]Please answer yes or no to the following questions:

Please choose the appropriate response for each item:

	Yes	Uncertain	No
Are absorbent pads used inside the packaging?	○	○	○
Is the packaging leak proof?	○	○	○
Is the packaging vacuum packed?	○	○	○
Is there any decontamination of the outside of the packaging?	○	○	○
At final packing does the company know which carcasses or chicken pieces are likely to be contaminated with Salmonella?	○	○	○
At final packing does the company know which carcasses or chicken pieces are likely to be contaminated with Campylobacter?	○	○	○
Are the carcasses that are known to be higher risk for containing salmonella treated any different from the rest?	○	○	○
Are the carcasses that are known to be higher risk for containing campylobacter treated any different from the rest?	○	○	○

[A78]What information does the label contain regarding safe handling, storage and cooking instructions?

Please write your answer here:

[A79]Which of the following methods are applied by the company to reduce risk to the consumer?

Check all that apply

Please choose all that apply:

- [ ] Freezing of carcasses followed by 31 days of storage at -20 degrees C
- [ ] Crust freezing using continuous carbon dioxide belt freezing of skinless breast fillets
- [ ] Other:

[FarmList]Please, can you attach a list of farms that supply the abattoir? Include a GPS location in the list. We will keep this list confidential. We will contact each farm and ask them to complete this questionnaire as well.

Please upload at most 5 files

Kindly attach the aforementioned documents along with the survey

Thank you for helping to improve human and poultry health in South Africa