Quantitative risk assessment of the likelihood of importing mechanically recovered poultry meat contaminated with *Salmonella* into South Africa

By

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MSc

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Declaration

I declare that “Quantitative risk assessment of the likelihood of importing mechanically recovered poultry meat contaminated with *Salmonella* into South Africa” is my work, and it has been done to fulfil a master’s degree in the field of paraclinical science. This work has never been submitted to any institution.

_____

Dr. T.N Ndobeni

May 2022

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Dedication

This dissertation is dedicated to my parents, Noxolo and Mlungisi Ndobeni and, my son Kagontle Ndobeni for giving me unwavering love and support.

“In everything give thanks; for this is the will of God in Christ Jesus concerning you”

1 Thessalonians 5:1

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Abstract

Mechanically recovered meat products have been linked to salmonellosis outbreaks in humans. However, no studies have investigated the prevalence and likelihood of mechanically recovered poultry meat (MRPM) imported to South Africa being contaminated with a *Salmonella* species; despite South Africa being a net importer of MRPM. This study seeks to address this gap by investigating the prevalence and factors associated with the likelihood of importing a *Salmonella*-contaminated consignment to South Africa. Furthermore, the study identified steps in the value chain likely to impact the levels of this likelihood significantly.

Secondary data of MRPM consignments imported through a South African port entry between 2016 and 2017 was used for this study. Crude and factor-specific proportions of *Salmonella* positive MRPM consignments and their 95% confidence intervals were calculated for all variables. A logistic regression model was fit to the data to assess the association between country of origin, importer, season, year, month, and MRPM *Salmonella* status. The likelihood of importing *Salmonella*-contaminated MRPM consignments was modelled using @Risk software in Microsoft Excel. Spearman's ranked correlation coefficients were used to assess the correlations between predictors and the likelihood of a consignment being contaminated with *Salmonella*.

A total of 8127 MRPM consignments were imported over the study period. Seventeen percent (17.18%, 985/5733) of the tested consignments were positive for *Salmonella* species. Among the 364 isolates that were serotyped, *Salmonella* Heidelberg (73.90%) was the most common serotype. The odds of consignment testing positive for *Salmonella* differed based on country of origin, season, and year of importation. The overall likelihood of importing *Salmonella*-contaminated MRPM

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consignment to South Africa was calculated as 5.88 $x10^{-4}$ (5^{th}= 5.886x 10^{-5}--95^{th}=5.73$x10^{-3}$) which was low. The prevalence of *Salmonella* in the exporting country (1.00) was significantly correlated with the likelihood of importing *Salmonella*-contaminated MRPM consignment to South Africa.

Salmonella species have been identified in MRPM consignments imported through a port of entry to South Africa. Among these, *Salmonella* Heidelberg was the most common. The quantitative risk assessment model suggests that there is likelihood of importing a positive consignment. However, some of the *Salmonella* serotypes reported in this study have been implicated in foodborne disease outbreaks. Therefore, a risk mitigation strategy must be developed, considering the *Salmonella* status of the exporting country, the importer, and the season of importation.

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List of Abbreviations

AMR	Antimicrobial Resistance
AI	Avian Influenza
CAC	Codex Alimentarius Commission
CAS	CHROMagar *Salmonella*
DALRRD	Department of Agriculture, Land Reform and Rural Development
EU	European Union
HPAI	Highly Pathogenic Avian Influenza
HIV	Human Immunodeficiency Virus
FAO	Food and Agriculture Organization
FSIS	Food Safety and Inspection Service
MRM	Mechanically Recovered Meat
MRPM	Mechanically Recovered Poultry Meat
MDR	Multi-drug Resistant
NTS	Non-typhoidal *Salmonella*
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
QRA	Quantitative Risk Assessment
QRAM	Quantitative Risk Assessment Model
SGSA	*Salmonella* Genoserotyping Array

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SAPA	South African Poultry Association
SOP	Standard Operating Procedure
TS	Typhoidal *Salmonella*
UK	United Kingdom
US	United States
USA	United States of America
VIP	Veterinary Import Permit
WHO	World Health Organization

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Chapter 1

1.1 Background

Salmonella is an anaerobic, rod-shaped, gram-negative bacteria known to cause salmonellosis in both animals and humans (Fagbamila et al., 2017). The organism is a commensal of the intestinal tract of humans, poultry, cattle, sheep, goats, and horses (Akpabio, 2015). *Salmonella* bacteria can be transmitted directly or indirectly by contacting or ingesting contaminated carcasses. In poultry, salmonellosis results in reduced growth, increased morbidity, and high mortality leading to economic losses for the farmers (Mund et al., 2017).

Salmonella species have been isolated in poultry and poultry products such as mechanically recovered meat (MRM). These sources have been implicated in human salmonellosis outbreaks (Akpabio, 2015; Ali & Sultana, 2016; Das et al., 2017). Mechanically recovered meat is obtained by pureeing carcasses left from bones, bone marrow, skin, and blood vessels, and the product has been noted to have good nutritional properties (Hecer & Sözen, 2011). The product has also been inexpensive, thus replacing red meat in the food industry (Hecer & Sözen, 2011).

Studies on the prevalence of *Salmonella* species in mechanically recovered poultry meat (MRPM) have been conducted in Poland (Modzelewska-Kapituła & Maj-Sobotka, 2014), United States of America (Taylor et al., 2015; Alali et al., 2016; Hutchinson, Wheeler & Mohle-Boetani, 2018), Europe (Kalaba et al., 2017), Brazil (Neves et al., 2018), Hungary (Tőzsér et al., 2019), and Ukraine (Martynenko & Rula, 2020). However, limited research has been conducted in South Africa.

Although salmonellosis is self-limiting in humans, clinical symptoms such as diarrhoea, nausea, vomiting, and abdominal pain have been reported six to 48 hours

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after exposure (Basler et al., 2016; Fagbamila et al., 2017). In addition, mortalities of up to 27% in children and up to 47% in adults have also been reported (Barbour et al., 2015).

Most *Salmonella* species are multi-drug resistant (MDR) (Eng et al., 2015) and the presence of resistance among the human isolates has been linked to exposure to antimicrobials (Keddy et al., 2009). Studies on antimicrobial susceptibility of *Salmonella* species affecting poultry and humans reported resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole (Eng et al., 2015). In South Africa antimicrobial resistance among *Salmonella* species to erythromycin, spectinomycin, oxytetracycline, streptomycin, doxycycline, sulphonamides, enrofloxacin, imipenem, tetracycline and trimethoprim-sulfamethoxazole have also been reported (Mokgophi et al., 2021; Ramatlala et al., 2021).

Clinical significance, economic losses, including health costs and human productivity loss due to work absence, have also been reported (Newell et al., 2010; Suijkerbuijk et al., 2016).

1.2 Justifications

Salmonella species have been reported as causes of illness in humans, with salmonellosis being among the most reported foodborne disease globally (Lekshmi et al., 2017). Furthermore, people with human immunodeficiency virus (HIV), malaria, and malnutrition especially in Africa are at a higher risk of *Salmonella* infection (Gordon, 2008; Feasay et al., 2021). Although *Salmonella* has been reported in poultry and poultry products (van Nierop et al., 2005), the role of imported MRPM in transmitting *Salmonella* has not been investigated in South Africa.

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1.3 Aim

This study aims to quantify the likelihood of importing MRPM consignment contaminated with *Salmonella* into South Africa and identify potential factors associated with the identified risk. The information generated from this study will contribute to the country's effort to develop a risk-based approach to importing and exporting animal products.

1.4 Objectives

The objectives of this study are as follows:

a) To investigate the prevalence and factors associated with importing a MRPM consignment contaminated with *Salmonella* into South Africa.

b) To estimate the likelihood of importing MRPM consignment contaminated with *Salmonella* into South Africa using the quantitative risk assessment (QRA) approach.

1.5 Benefits arising from this study

This study will help guide animal and public health policies on controlling and preventing *Salmonella* outbreaks in South Africa. Furthermore, this study can assist in developing a quantitative risk assessment framework for the importation of MRPM product in South Africa.

1.6 Ethics approval

This study was approved by the Department of Agriculture, Land Reform and Rural Development (DALRRD), Animal Ethics Committee, and Research Ethics Committee of the University of Pretoria (REC 182-19).

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1.7 Structure of the dissertation

This dissertation comprises five chapters. The first chapter provides the general background on the justification of the study, ethical approval, and structure of the dissertation. The second chapter reviews published studies on MRPM and *Salmonella* contamination. The third chapter investigated the prevalence and factors associated with *Salmonella* contamination in MRPM, the fourth chapter quantified the likelihood of importing MRPM contaminated with *Salmonella* into South Africa, and the fifth chapter comprises a summary, conclusions, and recommendations.

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Chapter 2

2.1 Literature Review

In communal areas, poultry farming has been practiced for many years as it provides income to those living below the poverty line (Guèye, 2000; Hecer & Sözen, 2011; Idowu, Mpayipheli & Muchenje, 2018). In recent years, global poultry consumption has increased tremendously as an inexpensive alternative source of protein (Farrell, 2013; Olobatoke & Mulugeta, 2015; Chauhan et al., 2017; Mottet & Tempio, 2017). As a result, many countries, including South Africa, have shifted from red meat to poultry meat consumption (EFSA, 2013). Mechanical recovered poultry meat (MRPM) is a product in South Africa, in which the demand has increased due to its affordability (BFAP, 2019). This shift has been attributed to the change in taste preference and reduced cost (Taha & Hahn, 2015).

However, the increase in accessibility of this affordable protein is likely to increase the exposure of a susceptible population to poultry pathogens including the foodborne pathogens associated with poultry products (Mayers, Mansfield & Brown, 2017). Moreover, MRPM has been linked to several human foodborne disease outbreaks (Antunes et al., 2016; Chauhan et al., 2017; Campos et al., 2018). *Salmonella*, *Campylobacter*, *Escherichia coli,* and *Listeria* are among the pathogens that have been reported as contaminants of poultry and poultry products (Bhaisare et al., 2014; EFSA, 2015; Rouger, Tresse & Zagorec, 2017); however, this study focuses on *Salmonella* organism (van Nierop et al., 2005; Dogru, Ayaz & Gencay, 2010; El Hussein et al., 2010; Kidanemariam, Engelbrecht & Picard, 2010). *Salmonella* contamination of food products is a major public health concern not only in South Africa but globally as well (Majowicz et al., 2010; Zdragas et al., 2012; Kagambèga et al.,

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2013; Rajagopal & Mini, 2013; Eng et al., 2015; Ali & Sultana, 2016; Tiwari & Bhatt, 2017; Marus et al., 2019; Hwang et al., 2020). Several studies have reported *Salmonella* in poultry and poultry products in South Africa (van Nierop et al., 2005; Dogru et al., 2010; El Hussein et al., 2010; Kidanemariam et al., 2010: Mokgophi et al., 2021; Ramatlala et al., 2021); however, limited information is available on the possibility of MRPM as a source of *Salmonella*.

2.2 *Salmonella* species aetiology, transmission, and clinical presentation

2.2.1 Aetiology

Salmonella belongs to the family *Enterobacteriaceae,* an anaerobic, rod-shaped, gram-negative bacteria known to cause salmonellosis in humans and animals (Fagbamila et al., 2017; Ryan, O'Dwyer & Adley, 2017; Hwang et al., 2020). There are two *Salmonella* species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* has been split into six subspecies; namely *Salmonella enterica* subspecies *enterica*, *salamae*, *arizonae*, *diarizona*, *houtenae,* and *indica* (Smith et al., 2007; CDC, 2013; Kemal, 2014; Smith, Seriki & Ajayi, 2016; Ryan et al., 2017). In addition, over 2500 serovars have been isolated from animals and humans (Pires et al., 2009; Zishiri, Mkhize & Mukaratirwa, 2016).

The majority of *Salmonella* species are host specific, with *Salmonella enterica* serotype Typhi and *Salmonella enterica* serotype Paratyphi (A and B) being associated with human salmonellosis. *Salmonella* Typhi and *Salmonella* Paratyphi are clinically associated with Typhoid fever, while the rest of the serovars are grouped as non-typhoidal *Salmonella* (NTS) (Tortora et al., 2004; Zou et al., 2014; Basler et al., 2016; Ryan et al., 2017; Johnson et al., 2018; Saleh et al., 2019; Kumar et al., 2022).

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Several serotypes of *Salmonella* have been reported in poultry, including *Salmonella* Entereditis, *Salmonella* Infantis, *Salmonella* Kentucky, *Salmonella* Heidelberg, *Salmonella* Pullorum, and *Salmonella* Gallinarum (Zishiri et al., 2016).

2.2.2 Transmission

Salmonella survives in a variety of conditions, including extremely low or high temperatures, dry environments for up to several months, and in soil for more than 332 days (Hanning, Nutt & Ricke, 2009; Jacobsen & Bech, 2012; Andino & Hanning, 2015; Wibisono et al., 2020). The bacterium transmission can be horizontal or vertical (Kallapura et al., 2014; Wibisono et al., 2020). The horizontal transmission occurs via the oral or aerosol route (Kallapura & Kogut et al., 2014). The oral route is the primary route of infection, while the aerosol route results from environmental contamination (Harbaugh et al., 2006; Scallan et al., 2011). The oral route of transmission is mainly through the consumption of contaminated poultry meat and poultry products (Magwedere et al., 2015; Akpabio, 2015; Antunes et al., 2016; Das et al., 2017; Firouzabadi et al., 2020), ingestion of contaminated water, and contact with the contaminated environment (Khan et al., 2010; Majowicz et al., 2010; Williams, Ebel & Hoeting, 2011; Marus et al., 2019). In addition, *Salmonella* species have been reported as a cause of zoonotic infection after contact with infected animals (Pires et al., 2009; Basler et al., 2015; Zishiri et al., 2016; Ryan et al., 2017; Johnson et al., 2018). Vertical transmission occurs due to a transovarian infection (Wibisono et al., 2020). This mechanism has been reported after a systemic infection (Kallapura et al., 2014).

2.2.3 Clinical signs in humans and poultry animals

In humans, salmonellosis is self-limiting; however, symptoms such as diarrhoea, vomiting, and abdominal pain have been reported (Antunes et al., 2016; Basler et al., 2016; Fagbamila et al., 2017). These symptoms can range from mild to

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moderate and last up to seven days. In addition, complications, including septicaemia and death, have also been reported (Majowicz et al., 2010; Cosby et al., 2015; Hao et al., 2016; Muvhali et al., 2017).

In poultry, the clinical presentation depends on the serotype involved. For example, in the case of *S.* Gallinarum and *S.* Pullorum clinical signs such as dehydration, reduced feed intake, anorexia, weakness, drooping, diarrhoea, and death have been reported (Kallapura et al., 2014).

2.3 *Salmonella* outbreaks in humans and poultry animals

2.3.1 Human outbreaks

Several human salmonellosis outbreaks associated with poultry and poultry products including MRPM have been reported (Ravel et al., 2009; Guo et al., 2011; Smith et al., 2014). These outbreaks have been reported in the United States (US), Brazil, Belarus, Bulgaria, Croatia, Denmark, Estonia, Finland, Germany, Greece, Japan, the Republic of Korea, Malaysia, the Philippines, and Thailand (Varma et al., 2005; Chittick et al., 2006; Hendriksen et al., 2011; Loharikar et al., 2012; Tondo & Ritter, 2012; Basler et al., 2015; Basler et al., 2016; Marlow et al., 2017; Hutchinson, Wheeler & Mohle-Boetani, 2018; Finger et al., 2019). Similarly, human outbreaks have also been reported in African countries including Cameroon, Tunisia, Senegal, and Nigeria (Fashae et al., 2010; Hendriksen et al., 2011) and South Africa (Smith et al., 2007; GERM-SA, 2010, 2011, 2012; Barbour et al., 2015; Magwedere et al., 2015; Olobatoke & Mulugeta, 2015; Zishiri et al., 2016; Motladiile et al., 2016; Muvhali et al., 2017).

South Africa has also reported an increase in human clinical cases of both typhoidal *Salmonella* (TS) and non-typhoidal *Salmonella* (NTS) between 2006 to 2017

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(Oloso, et al., 2017) with NTS cases being rarely reported (Barbour et al., 2015; GERMS-SA, 2016, 2017). Overall, under-reporting of *Salmonella* infections remains a challenge in South Africa (Olobatoke & Mulugeta, 2015; Oloso et al., 2017). In 2004, a study conducted in the Gauteng province reported that poultry carcasses entering the food chain were contaminated with *Salmonella* species (van Nierop et al., 2005). In addition, NTS foodborne infections associated with the consumption of poultry products have also been reported in other parts of the country (Smith et al., 2007; Majowicz et al., 2010; Olobatoke & Mulugeta, 2015). It is possible that incidence of human outbreaks could be higher than reported due to lack of pathogen surveillance systems, limited documentation, and under reporting of clinical cases (CDC, 2006).

2.3.2 Animal Outbreaks

Poultry outbreaks associated with *S.* Gallinarum, *S.* Pullorum, and *S.* Entereditis have been widely reported (Mahajan & Rakha, 2010; Taddele, Rathore & Dhama, 2012; Kumar, Rajagopal & Mini, 2013; Adamu et al., 2017). These outbreaks have been reported in India, Brazil, Korea, France, Spain, Italy, Germany, Denmark, Russia, Argentina, and China (Kumar et al., 2010; Dey et al., 2016; De Carli et al., 2017; Celis-Estupiñan et al., 2017; Lee et al., 2020). In Africa, fewer *Salmonella* outbreaks have been reported in Nigeria and Ethiopia (Ezema, Onuoha & Chah, 2009; Mbuko et al., 2009; Barrow & de Freitas, 2011; Mamman et al., 2014; Adamu et al., 2017).

2.4 Risk factors for *Salmonella* importation and infection

2.4.1 Risk of importation

Several risk factors have been associated with the likelihood of importing MRPM contaminated with *Salmonella*. These include country of origin, season, and

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year (Rose et al., 2002; Ghafir et al., 2005; Zdragas et al., 2012; Rania & Qasem, 2014; Kramarenko et al., 2014; Campos et al., 2018; Hutchinson et al., 2018 Regalado-Pineda et al., 2020; Ndobeni, Magwedere & Qekwana, 2022). In addition, hygiene practices, biosecurity, disease control, and farming practices have also been associated with an increased risk of importing *Salmonella* (Zishiri et al., 2016; Akramzadeh et al., 2020). In view of this, the country's policy seems to play a significant role in the likelihood of importing and exporting MRPM contaminated with a *Salmonella* species (Campos et al., 2018; Akramzadeh et al., 2020).

2.4.2 Risk of human infection

Risk for human infection is often associated with undesirable behavioural patterns including inadequate hygiene, the lack of the usage of non-adsorbent chopping board, consumption of raw and undercooked poultry meat and poultry products (Mughini-Gras et al., 2014). Furthermore, the incidence of the disease seems to depend on the season, age, immune status, location, history of international travel, and association with animals (Hendriksen et al., 2009; Varga et al., 2012; Varga et al., 2013; Crump et al., 2015; Muvhali et al., 2017).

In poultry, *Salmonella* infections are common in stressed, immunocompromised, young, and old birds (Crump et al., 2015). In addition, seasonality, housing environment, contaminated feed or water, and poultry handling have been associated with an increased risk of infection in poultry animals (van Hoorebeke et al., 2010; Volkova et al., 2011). In Zambia, poor hygiene practices in slaughterhouses were identified as a risk factor for carcass contamination with *Salmonella* (Heyndrickx et al., 2002; Haileselassie et al., 2013; Manyori et al., 2017).

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2.5 Antimicrobial resistance among *Salmonella* species

The use of antimicrobials to prevent and treat bacterial infections in food animals started has a long history (Hao et al., 2016). Antimicrobials have been used to improve feed efficiency and promote animal growth (Dibner & Richards, 2005). The increase in food demand due to population growth is likely to exert pressure on poultry industries to use more antimicrobials (Zishiri et al., 2016). Recently, there are increasing reports of misuse of antimicrobials such as fluoroquinolones, macrolides, cephalosporins, and tetracyclines in humans and animals. The misuse of antimicrobials is among the primary drivers of AMR (Cosby et al., 2015; Fletcher, 2015; Hao et al., 2016; Xiong et al., 2018). In addition, some antimicrobials used in animals have a similar mechanism of resistance to those used in humans (Hao et al., 2016). The increasing prevalence of antimicrobial resistance has been recognized by World Health Organization (WHO) as an essential threat to public health (Xiong, Sun & Zeng, 2018).

2.6 Mechanically recovered meat

2.6.1 International legislation

The biggest exporters of poultry meat and poultry products into South Africa are Brazil, European Union (EU), and the US (Makgopa & Bonsu, 2020). The Commission Regulation (EC) No 2073/2005 of 15 states that MRPM consignments must have zero samples testing positive for *Salmonella* species (EU, 2014; Jansen et al., 2018). In the US, the Food Safety and Inspection Service (FSIS) accepts five out of 51 samples to test positive for *Salmonella* (Ollinger & Taha, 2015).

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2.6.2 South African legislation

In South Africa, all importers are required to apply for an import permit with DALRRD. The South African government then evaluates the risk of importing a commodity into the country based on the disease status of the exporting country, among other factors. Additional information may be requested on veterinary control, surveillance programs, and food safety measures (DAFF, 2008). Once the risk is determined to be negligible in line with the Animal Disease Act 35 of 1984 and the Meat Safety Act 40 of 2000, a certificate of importation is issued, which is valid for six months. This certificate can only be used once for one consignment. During importation, the veterinary health certificate from the veterinary authority of the exporting country must accompany the consignment into the country. On arrival in the country, each consignment of the MRPM is tested for microbiological contamination to assess its risk to the human or animal population in South Africa (DAFF, 2008).

2.6.3 Importation of meat and meat products

In 2017, seventy five percent (75%) of meat imported into South Africa was poultry and poultry products. This was attributed to an increase in local demand for poultry meat (DAFF, 2017). Among the imported poultry and poultry products was the inexpensive MRPM used as a source of cheap animal protein in further processing (Hecer & Sözen, 2011; Farrell, 2013; Olobatoke & Mulugeta, 2015; Chauhan et al., 2017; Mottet & Tempio, 2017). In the first quarter of 2019, MRPM importation was 40.5% of the imported poultry consignments (SAPA, 2019).

2.6.4 Distribution of mechanically recovered poultry meat

Imports of MRPM to South Africa comes from countries such as Spain, Canada, Denmark, Thailand, Brazil, US, Belgium, Ireland, Argentina, United Kingdom (UK), and Australia (SAPA, 2017, 2018, 2019) with Brazil being the main exporter based on

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quantity. In year 2017, the import from Brazil has gradually increased from 48.8% to 65.1% (SAPA, 2017).

2.7 Import risk analysis

The growing trade of meat products will likely increase the global spread of infectious diseases (Evans & Leighton, 2014; Antunes et al., 2016) and to mitigate the risk of *Salmonella* contamination associated import of meat and meat products, a value chain approach is needed. Risk analysis is a method or tool that can be used to assess the likelihood of importing pathogens through live animals or animal products (Hald et al., 2004; Hoffmann, 2010; Peeler, Reese & Thrush, 2015; Vose, 2008, 1997).

Risk analysis has been internationally recognized as an effective and holistic approach to reducing foodborne disease outbreaks through assessment of the likelihood of importing pathogens through live animals or animal products (Zwietering & van Gerwen, 2000; FAO & WHO, 2003; Lammerding & Fazil, 2000; Lammerding, 2006; Mead et al., 2010; Hoffmann, 2010; Qekwana, McCrindle & Oguttu, 2012; Peeler, Reese & Thrush, 2015). Import risk analysis characterized as qualitative or quantitative has been used in several studies (Lammerding & Fazil, 2000; Hoffmann, 2010; Peeler, Reese & Thrush, 2015; OIE, 2021) such as Canada, Denmark, Australia, New Zealand, UK, and China (Hald et al., 2004; Dong et al., 2015; Peeler, Reese & Thrush, 2015).

In qualitative risk analysis, likelihood, hazard exposure, and magnitude consequence prioritizes the identified risks using a pre-defined rating scale usually expressed non-numerically as low or negligible, medium, and high (Bruckner et al., 2010). However, quantitative risk analysis numerically represents the likelihood and exposure to a hazard. In addition, uncertainty and variability are modelled by

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probability distributions (Lammerding & Fazil, 2000; Zwietering & van Gerwen, 2000; FAO & WHO, 2003; Lammerding, 2006; EFSA, 2008; van Hoorebeke et al., 2010; Mead et al., 2010; Oscar, 2011; Peeler et al., 2015). In this study, a quantitative risk analysis approach was performed.

2.7.1 Hazard identification

Hazard identification is the primary step required to recognize a potential problem or concern to be investigated (Cassin et al., 1998; Lammerding & Fazil, 2000; OIE, 2021). In import risk analysis, a hazard can be a pathogenic agent that could result in economic or public health adverse effects (FAO & WHO, 2009). In this study, *Salmonella* species was identified as an essential hazard known to cause diseases in humans and poultry (Lammerding & Fazil, 2000; Qekwana, McCrindle & Oguttu, 2012). Studies on the potential introduction of *Salmonella* species and unique specific serotypes via imported MRPM have not been done (Peeler, Reese & Thrush, 2015).

2.7.2 Risk assessment

Risk assessment can be qualitative or quantitative; for this study, a quantitative risk assessment (QRA) approach was used for characterizing the variability and uncertainty of the risk (Schlundt, 2000; Lammerding & Fazil, 2000; Zwietering & van Gerwen, 2000; FAO & WHO, 2003; Lammerding, 2006; EFSA, 2008; Mead et al., 2010; Oscar, 2011; OIE, 2021). Quantitative risk assessment is a mathematical approach used to estimate the likelihood and severity of a risk associated with a specified hazard (Schlundt, 2000; Lammerding & Fazil, 2000; Oscar, 2011; Dong et al., 2015). This approach identifies microbial pathogen safety at different stages of the value chain that causes the risk of foodborne diseases (Cassin et al., 1998). Import risk assessment is divided into entry assessment, exposure assessment, consequence assessment, and risk estimation (Zwietering & van Gerwen, 2000; Kelly

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et al., 2003; Oscar, 2011; Kalaba et al., 2017; OIE, 2021). This study used QRA to estimate the likelihood of importing MRPM contaminated with *Salmonella* through a port of entry into South Africa.

2.7.2.1 Entry assessment

Entry assessment is an assessment of how a pathogen is introduced into an importing country and the likelihood of such introduction. In this current study, the entry assessment component involves the following inputs: biological factors such as the type of species or serotype, testing at the port of entry; occurrence of *Salmonella* species as a hazard in South Africa, veterinary inspection service at the port of entry, *Salmonella* species testing, a report for compliance status of the MRPM product, and control measures implemented by the exporting country (DAFF, 2008; Kuznetsova, 2016; OIE, 2021).

2.7.2.2 Exposure assessment

Exposure assessment describes how humans and animals are exposed to the hazard in the importing country and estimates the likelihood of the exposure occurring. Concerning this study, the inputs for the likelihood of exposure are biological properties of *Salmonella* species, importing country factors such as the presence of the *Salmonella* species bacteria, environmental attributes, human and animal demographics, custom practices, and MRPM consignment factors (Kuznetsova, 2016; OIE, 2021).

2.7.2.3 Consequence assessment

Consequence assessment determines the adverse effects of a hazard in animals, and public health as well as economically (Lammerding & Fazil, 2000; Peeler, Reese & Thrush, 2015; Kuznetsova, 2016; OIE, 2021). Adverse animal and human

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health effects including morbidities and mortalities have been reported (Majowicz et al., 2010; Cosby et al., 2015; Hao et al., 2016; Muvhali et al., 2017). Additionally, animals with salmonellosis have reduced feed intake and are often anorexic (Kallapura et al., 2014). Economic adverse effects reported have been mainly associated with the cost of disease surveillance investigation, treatment, and international trade restrictions (USDA, 2003; Suijkerbuijk et al., 2016).

2.7.2.4 Risk estimation

Risk estimation integrates entry, exposure, and consequence assessment to estimate the overall risk (Cassin et al., 1998; Lammerding & Fazil, 2000; Kuznetsova, 2016; OIE, 2021). These include integration probability distributions, confidence intervals to express uncertainties, and correlation between the model input variable and the level of risk identified (OIE, 2021).

2.7.3 Risk management

Risk management involves evaluating the identified risk based on the described scenario (Qekwana, McCrindle & Oguttu, 2012; Kuznetsova, 2016; OIE, 2021). It considers risk assessment as a component that provides scientific evidence in the presence of uncertainty (Hoffmann, 2010; Qekwana, McCrindle & Oguttu, 2012). Regulatory authorities prescribe the identified control measures to be implemented by relevant stakeholders. Control measures are then implemented to manage and reduce the risk of importing *Salmonella*-contaminated MRPM consignment into South Africa (DAFF, 2000, 2006). At all stages, monitoring and review must ensure that the proposed measures are still effective in reducing the risk (Qekwana, McCrindle & Oguttu, 2012; OIE, 2021).

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2.7.4 Risk communication

Sanitary risk communication is where information and scientific opinion about the hazard is translated and communicated via several avenues, such as policymakers, scientists, consumers, academia, regulatory, and agencies (Lammerding & Fazil, 2000; Lammerding, 2006; Kuznetsova, 2016; OIE, 2021). Communication on the risk of importing *Salmonella*-contaminated MRPM into South Africa may involve sharing relevant empirical information between exporting and importing countries, domestic livestock producers, and consumer groups (Qekwana, McCrindle & Oguttu, 2012). Peer review and community awareness programs can also be used (Tiozzo et al., 2011; Qekwana, McCrindle & Oguttu, 2012; Tambo, Yah & Madjou, 2018; OIE, 2021).

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Chapter 3

Prevalence and factors associated with *Salmonella* species contamination of mechanically recovered poultry meat imported in South Africa, 2016-2017.

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3.1 Abstract

Mechanically recovered meat products have been linked to outbreaks of human salmonellosis. However, there are no studies that have investigated the prevalence of *Salmonella* species in mechanically recovered meat products in South Africa despite most mechanically recovered meat products being imported. Therefore, this study investigated the prevalence and factors associated with *Salmonella* contamination in mechanically recovered poultry meat imported into South Africa.

This study used secondary data of mechanically recovered poultry meat consignments imported through a port entry in South Africa between 2016 and 2017. Crude and factor-specific proportions of *Salmonella* contamination in mechanically recovered poultry meat and their 95% confidence intervals were calculated. For assessment of the association between exploratory variables, country of origin,

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season, importers, year, and mechanically recovered poultry meat *Salmonella* status, a logistic regression model was used.

A total of 8127 consignments of mechanically recovered poultry meat were imported in this study. Seventeen percent (17.18%, 985/5733) of consignments were contaminated with *Salmonella* species and only 364 serotypes were isolated. *Salmonella* Heidelberg (73.90%) was the most common serotype followed by *Salmonella* Infantis (6.59%), *Salmonella* Salamae (4.67%), and *Salmonella* Schwarzengrund (3.57%). The odds of testing positive for *Salmonella* was higher amongst consignments from country-B (OR: 3.958, $p < 0.0001$) compared to "All others". The odds of testing positive for *Salmonella* was also higher among consignments imported in autumn (OR: 1.488, $p < 0.0001$) but lower among those imported in spring (OR: 0.767, $p = 0.0004$) and summer (OR: 0.843, $p < 0.0001$) when compared to the winter season. Consignments imported in 2016 compared to 2017 were 1.563 times ($p < 0.0001$) as likely to test positive for *Salmonella* species.

Salmonella species have been reported in mechanically recovered poultry meat imported through a port of entry to South Africa. Among the reported species, *Salmonella* Heidelberg was the most common. Since some of the *Salmonella* species reported in this study have been implicated in foodborne disease outbreaks. An import risk mitigation for *Salmonella* contamination strategy must be developed taking into consideration country of origin, importer, and season of importation.

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3.2 Background

Mechanically recovered meat is a product attained from separating meat from bone (Hecer & Sözen, 2011; EFSA, 2013; Chauhan et al., 2017). The increased consumption of poultry meat in South Africa (Olobatoke & Mulugeta, 2015) has made MRPM from poultry an inexpensive alternative source of protein (Hecer & Sözen, 2011; Chauhan et al., 2017). Mechanically recovered meat is used for the production of a variety of products including mince, chunks, and soup packs, and most of it is imported in South Africa (BFAP, 2019). Despite reports of *Salmonella* species contamination of poultry and poultry products in South Africa (van Nierop et al., 2005; Dogru et al., 2010; El Hussein et al., 2010; Kidanemariam et al., 2010), the possibility of MRPM as a source of salmonellosis has not been investigated.

Salmonella species are anaerobic, rod-shaped, gram-negative bacteria belonging to either *Salmonella bongori* or *Salmonella enterica* species group (Fagbamila et al., 2017). Although the genus *Salmonella* affects the intestinal tract of humans and animals, it has been reported as a cause of salmonellosis cases in humans and animals (Akpabio, 2015). Most *Salmonella* species are host specific with *Salmonella* Typhi and *Salmonella* Paratyphi associated with human infections while *Salmonella* Pullorum and *Salmonella* Gallinarum common in avian species (Tortora *et al.*, 2018).

Several *Salmonella* species have been reported in foodborne illness (Lekshmi et al., 2017) and persons with underlying clinical conditions are at a higher risk of infection (Gordon, 2008). Humans get infected with *Salmonella* through ingestion of contaminated food products or indirectly through a contaminated environment (Sigaúque et al., 2009; Khan et al., 2010; Majowicz et al., 2010; Akpabio, 2015; Ali & Sultana, 2016; Das et al., 2017). However, most human outbreaks are associated with

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the consumption of poultry products (Herikstad et al., 2000; Wilson, 2007; Sigaúque et al., 2009; Alali et al., 2012; Meldrum, Olobatoke & Mulugeta, 2015).

In most cases, salmonellosis is self-limiting with reported clinical signs including diarrhoea, nausea, vomiting, and abdominal pain (Basler et al., 2016; Fagbamila et al., 2017). However, mortality of up to 27% in children and up to 47% in adults (Barbour et al., 2015), increased health costs, and loss of human productivity have been reported in severe cases of salmonellosis (Newell et al., 2010; Suijkerbuijk et al., 2016). The high mortality has been attributed to among others, high levels of resistance to commonly used antimicrobials which impacts patient care and prognosis (Keddy et al., 2009; Eng et al., 2015).

This study investigated the prevalence of *Salmonella* species in MRPM imported into South Africa between 2016 and 2017. Furthermore, this study investigated factors associated with the likelihood of importing a consignment contaminated with *Salmonella* into South Africa. The information generated from this study will be used for guidance of animal and public health policies on the control and prevention of *Salmonella* outbreaks in South Africa. Furthermore, this study can be used to develop a quantitative risk assessment framework for the importation of MRPM into South Africa.

3.3 Materials and Methods

3.3.1 Ethics approval

This study was approved by the DALRRD and the Animal Ethics and Research Ethics Committees of the University of Pretoria (REC 182-19).

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3.3.2 Data collection

The South African government evaluates the risk of importing a commodity into the country based on among others the disease status of the exporting country, answers to a questionnaire, inspection visits, disease control, and surveillance programs (DAFF, 2008). Once the risk is determined to be negligible in line with the Animal Disease Act 35 of 1984, and the Meat Safety Act 40 of, the country is approved, and specific establishments are listed as eligible for export of MRPM to South Africa.

All importers are required to apply for an import permit with DALRRD. The imported consignment is accompanied by the veterinary health certificate issued by the exporting country's veterinary authority. A Veterinary Import Permit (VIP) is to be used only once and it is valid for six months. Upon arrival at the port of entry in South Africa, each consignment of the MRPM undergoes documentary inspection, and physical inspection followed by a determination of the level of microbiological contamination of the product to assess its risk to human or animal populations in South Africa (DAFF, 2008).

Under the Meat Safety Act 40 of 2000, microbiological testing is mandatory as part of compliance monitoring. On arrival and at the cold storage, five samples from selected consignment packages are randomly collected and placed in labelled sterile sample bags for further laboratory analysis. At the laboratory, a minimum of 25g is used for the identification of *Salmonella* species followed by serotyping in the case of a positive test. The results of the bacteriological tests are then captured in the laboratory database and a report as to the compliance status of the product is issued to the Veterinary Inspector at the port of entry (DAFF, 2008).

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3.3.3 Data source and preparation

Laboratory records of *Salmonella* results of MRPM imported into South Africa from 2016 to 2017 were included in this study. The following fields were extracted from the database country of origin, *Salmonella* results status, date of sampling, and importer. The dataset was assessed for missing values and incomplete information. None were observed in this dataset.

In total, 16 countries exported MRPM via the selected port of entry. However, 14 countries had exported less than 1% of consignments and were re-categorized as "All others". Similarly, importers who imported <1% of consignments via the selected port of entry were re-categorized as "All others". The variable "Season" was categorized into summer (November – March), autumn (April – May), winter (June – August), and spring (September – October).

3.3.4 Data analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) statistical package. Crude and factor-specific proportions of *Salmonella* contamination in MRPM, as well as their 95% confidence intervals, were calculated. Associations between categorical variables; country, month, season, year, importer, and MRPM *Salmonella* status were assessed using the Chi-square or Fisher's Exact tests where appropriate. The significance of all statistical tests was set at $\alpha = 0.05$ for all the above tests.

3.3.5 Predictors of *Salmonella* contamination

A logistic regression model was used to assess the predictors of *Salmonella* in MRPM. A univariable logistic regression model was used to assess the association between exploratory variables, country, season, importer, year, and MRPM

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Salmonella status. The predictors with a *p*-value less than 0.20 were considered for inclusion in the multivariable logistic regression model.

A multivariable logistic regression model using backward selection was fitted to the data containing all categorical variables with potential association ($p \leq 0.2$) with the outcome. The significance of the predictor variables was set at $\alpha = 0.05$. A comparison of change in model coefficients with and without suspected confounders was used to assess confounding. If a suspected confounding variable was removed resulting in a 20% or greater change in another model coefficient, the variable removed was considered a confounder and remained in the model irrespective of its statistical significance. Adjusted odds ratios and 95% confidence intervals were calculated for all four predictors retained in the final model. Hosmer-Lemeshow goodness of fit test was used to assess the goodness of fit of the final model.

3.4 Results

In total 8127 consignments were imported, of these, 5733 (70.54%) were tested for *Salmonella* species and 985 (17.18%, 95% CI: 16.23-18.18) tested positive for *Salmonella* species. The majority of *Salmonella* positive consignments came from Country B (26.95%) followed by Country A (16.89%). Importer-VI (26.10%) had the highest proportion of consignments testing positive, followed by importer-IV (18.80%). The year 2016 (21.12%) had more consignments positive for *Salmonella* species compared to the year 2017 (14.45%). The proportions of consignment testing positive for *Salmonella* also differed depending on the season **(Table 3.1)**.

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Table 3.1: Proportion of *Salmonella*-contaminated consignments imported through a port of entry in South Africa between 2016 and 2017.

	Consignments tested			*Salmonella* positive		
	n[a]	%	95% CI[b]	n[c]	%	95% CI[b]
Country						
Country A	5125	89.39	88.57-90.17	866	16.89	19.15-21.57
Country B	319	5.56	5-6.18	86	26.95	30.97-43.27
All others	289	5.04	4.50-5.63	33	11.41	9.32-17.55
Importer						
I	2285	40.12	38.86-41.41	365	15.97	14.53-17.53
II	828	14.54	13.65-15.48	97	11.70	9.69-14.08
III	559	9.81	9.07-10.62	73	13.05	10.52-16.11
IV	449	7.88	8.05-9.52	79	18.80	12.89-19.29
V	440	7.72	7.06-8.45	42	9.54	7.14-12.65
VI	295	5.18	4.63-5.78	77	26.10	21.42-31.4
VII	220	3.86	3.39-4.39	31	14.09	10.11-19.31
All others	352	6.04	5.42-6.64	99	28.12	23.68-33.04
Month						
January	280	4.77	4.25-5.35	20	7.14	4.67-10.77
February	283	4.83	4.30-5.40	29	10.24	7.23-14.33
March	427	7.28	6.64-7.97	68	15.92	12.76-19.7
April	257	4.38	3.88-4.93	54	21.01	16.48-26.42
May	223	3.80	3.34-4.32	45	20.17	15.44-25.93
June	489	8.34	7.65-9.07	54	11.04	8.56-14.13
July	591	10.08	9.33-10.87	104	17.59	14.74-20.87
August	787	13.42	12.57-14.31	162	20.58	17.91-23.55
September	910	15.52	14.61-16.46	174	19.12	16.7-21.8
October	687	11.71	10.92-12.56	101	14.70	12.25-17.55
November	518	8.83	8.13-9.58	136	26.25	22.65-30.21
December	413	7.04	6.41-7.72	84	20.33	16.74-24.49
Year						
2016	2746	46.82	45.55-48.1	580	21.12	19.64-22.69
2017	3119	53.18	51.9-54.45	451	14.45	13.27-15.74
Season						
Autumn	480	8.18	7.50-8.91	99	20.62	17.25-24.47
Spring	1597	27.23	26.11-28.38	275	17.21	15.45-19.15
Summer	1921	32.75	31.56-33.97	337	17.54	15.91-19.31
Winter	1867	31.83	30.65-33.04	320	17.13	15.5-18.92

[a] **Number of consignments tested**
[b] **Confidence Interval**
[c] **Number of *Salmonella* positive consignments**

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Over the study period, there were monthly variations in the proportions of MRPM consignments contaminated with *Salmonella* **(Figure 1)**. In addition, there was a slight increase ($p < 0.0001$, Adjusted R^2=0.0039) in the monthly proportions of *Salmonella*-contaminated consignments.

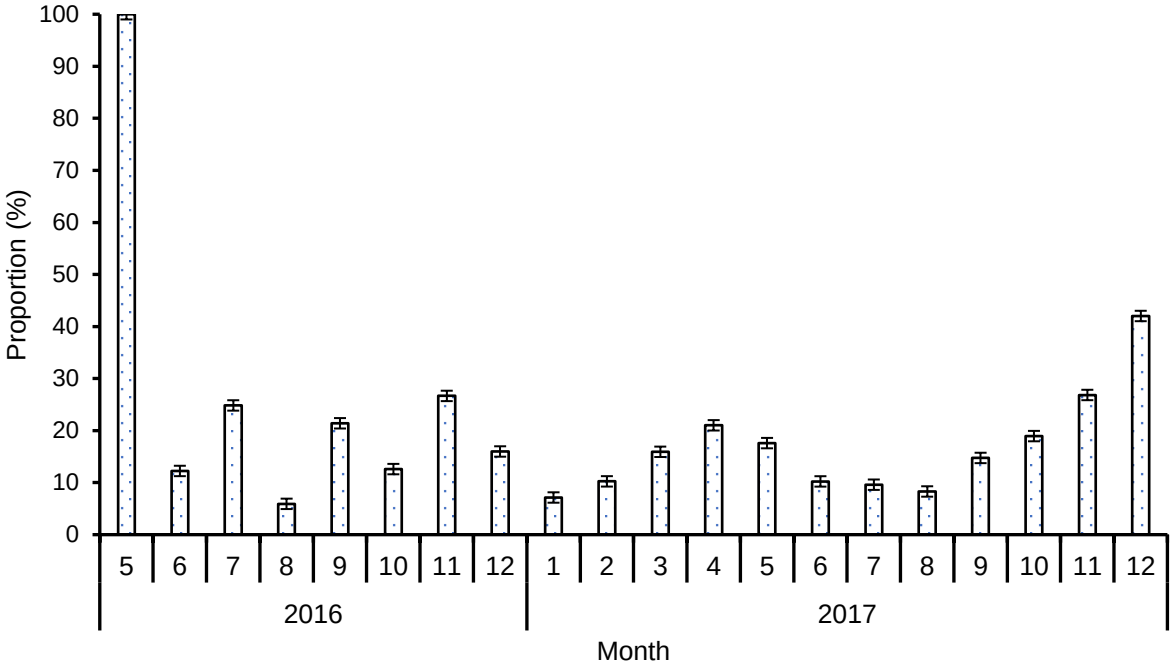

Figure 3.1: Monthly distribution in the proportions of consignments contaminated with *Salmonella* species between 2016 and 2017.

Three hundred and sixty-four (364) of the *Salmonella* isolates were serotyped. Of these, *Salmonella* Heidelberg (73.90%) was the most common followed by *Salmonella* Infantis (6.50%), *Salmonella* Salamae (4.67%), and *Salmonella* Schwarzengrund (3.57%) **(Table 3.2)**.

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Table 3.2: Distribution of *Salmonella enterica* serotypes reported from consignments imported in South Africa between 2016 and 2017.

Serotype	Frequency	Percent
Salmonella Heidelberg	269	73.90
Salmonella Infantis	24	6.59
Salmonella Salamae	17	4.67
Salmonella Schwarzengrund	13	3.57
Salmonella Enteritidis	6	1.65
Salmonella Minnesota	6	1.65
Salmonella Muenchen	5	1.37
Salmonella Rough	5	1.37
Salmonella Coromandel	4	1.10
Salmonella Saintpaul	3	0.82
Salmonella Virginia	3	0.82
Salmonella Abony	1	0.27
Salmonella Brancaster	1	0.27
Salmonella Derby	1	0.27
Salmonella Fillmore	1	0.27
Salmonella Gloucester	1	0.27
Salmonella Irumu	1	0.27
Salmonella Tshiongwe	1	0.27
Salmonella Typhimurium	1	0.27
Salmonella Worthington	1	0.27

3.4.1 Predictors of *Salmonella* species among the consignments

Based on the univariable logistic regression model, country ($p < 0.0001$), importer ($p = 0.0001$), season ($p < 0.0001$), and year ($p = 0.0001$) were potential predictors of a consignment to be contaminated with *Salmonella* species at a liberal $\alpha \leq 0.20$. Therefore, they were included in the multivariable model.

In the final model, the odds of contamination for *Salmonella* species were significantly higher among consignments from country-B (OR: 3.958, $p < 0.0001$) compared to “All others”. Consignments imported in 2016 were 1.563 times ($p < 0.0001$) as likely to be contaminated with *Salmonella* when compared to consignments imported in 2017. Seasonally, the odds of contamination for *Salmonella* were higher

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in consignments imported in autumn (OR: 1.488, $p < 0.0001$) but lower in summer (OR: 0.843, $p < 0.0001$) and spring (OR: 0.767, $p = 0.0004$) when compared to the winter season. Importer-V (OR: 0.238, <0.0001) had lower odds of importing a consignment contaminated with *Salmonella* species compared to "All others". While the odds of importing a *Salmonella*-contaminated consignment was higher among those imported by importer-VI (OR: 0.461, <0.0001) compared to "All others" **(Table 3.3)**.

Table 3.3: Univariable and multivariable logistic models showing predictors of *Salmonella* among MRPM consignments imported between 2016 and 2017.

	Univariable logistic regression				Multivariable logistic regression			
	OR[a]	95% CI[b]		*p*-value	OR[a]	95% CI[b]		*p*-value
Country				<0.0001				
A	1.577	1.090	2.283		1.879	1.255	2.812	0.6743
B	2.863	1.846	4.441		3.958	2.421	6.470	<0.0001
All others	Ref	-	-		Ref	-	-	-
Season				<0.0001				
Autumn	1.256	0.977	1.616		1.488	1.124	1.969	<0.0001
Spring	1.006	0.842	1.200		0.767	0.629	0.935	0.0004
Summer	1.029	0.869	1.217		0.843	0.698	1.018	<0.0001
Winter	Ref	-	-		Ref	-	-	-
Importer				0.0001				
VI	1.476	1.056	2.064		1.110	0.933	0.792	<0.0001
II	0.555	0.412	0.749		0.471	0.350	0.634	0.0706
I	0.420	0.285	0.619		0.657	0.519	0.832	0.0553
IV	0.768	0.604	0.977		0.461	0.323	0.658	0.1114
III	0.531	0.330	0.853		0.544	0.394	0.751	0.6792
VII	0.637	0.454	0.893		0.558	0.360	0.864	0.8862
V	0.593	0.427	0.825		0.238	0.160	0.355	<0.0001
All others	Ref	-	-		Ref	-	-	-
Year				<0.0001				
2016	1.584	1.383	1.814		1.677	1.414	1.989	<0.0001
2017	Ref	-	-		Ref	-	-	-

[a] **Odds Ratio**
[b] **Confidence interval**

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3.5 Discussion

Salmonellosis remains a major public health concern globally (Majowicz et al., 2010), thus, research on MRPM as a potential source is critical in guiding risk mitigation strategies. Moreover, NTS species have been associated with 600 million diarrhoeal cases globally (WHO, 2017) and significantly contribute to high incidences of foodborne illnesses in South Africa (Muvhali et al., 2017; Keddy et al., 2018).

In the present study, the proportion of *Salmonella*-contaminated MRPM consignments was similar to the 15% that was reported in California (Hutchinson et al., 2018) and 13% reported in Belgium (Ghafir et al., 2005). In contrast, higher prevalences of *Salmonella* species in MRPM have been reported in the US (44.6%) (Rose et al., 2002) and Estonia (38.5%) (Kramarenko et al., 2014). While a study in Jordan identified no *Salmonella* species from MRPM (Rania & Qasem, 2014). The presence of *Salmonella* species in this study suggests the risk of importing MRPM contaminated with *Salmonella* species exists (Campos et al., 2018). Since, South Africa imports almost all of its MRPM (Zishiri et al., 2016), a QRA must be implemented to evaluate the current mitigation strategy and make recommendations for effective monitoring and surveillance. Moreover, in the likely event of a breakdown in animal and food safety mitigation measures, this may lead to outbreaks of salmonellosis in South Africa (Rose et al., 2002; Hecer & Sözen, 2011; Olobatoke & Mulugeta, 2015; Akramzadeh et al., 2020).

The serotypes reported in this study from *Salmonella enterica* are known to be zoonotic and have global public health concerns (Knodler & Elfenbein, 2019; Motladiile *et al.*, 2019). For example, *Salmonella* Heidelberg has been reported in 96% of inmates who consumed MRPM in the USA (Taylor et al., 2015). In addition,

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Salmonella Schwarzengrund and *Salmonella* Infantis have also been isolated from MRPM in the USA (Alali et al., 2016) and *Salmonella* Enteriditis in 9% of MRPM in Brazil (Neves et al., 2018). Therefore, the results of this study further demonstrate the need to evaluate current regulations relating to the import of MRPM into South Africa.

The South African regulations of Meat Safety Act 40 of 2000 on poultry and poultry products require that biological hazards be identified, prevented, eliminated, or reduced to an acceptable level (DAFF, 2000, 2006). However, the South African SOP allows for the importation of commodities positive for *Salmonella* serotypes except for *Salmonella* Typhi*, Salmonella* Enteriditis, and *Salmonella* Typhimurium (DAFF, 2011). Furthermore, this operating procedure allows for the importation of commodities provided that no more than two out of five samples tested positive for *Salmonella* species. This contradicts the above-mentioned regulations that stipulate that for a consignment to be accepted in South Africa no pathogen must be detected. In view of the fact, *Salmonella* serotypes known to cause diseases in humans have been isolated from imported MRPM consignments, the current SOP must be updated to align with the regulation of the Meat Safety Act 40 of 2000 (Hoffmann et al.,2013).

The biggest exporters of poultry meat and poultry products to South Africa are Brazil, EU, and the US (Makgopa & Bonsu, 2020)**.** In the EU, rejections of poultry products are mostly associated with *Salmonella* contamination (Jansen et al., 2016). The Commission Regulation (EC) No 2073/2005 of 15 states that MRPM consignments must have zero samples testing positive for *Salmonella* species (EU, 2014; Jansen et al., 2018). In the US, the FSIS accepts five out of 51 samples to test positive for *Salmonella* (Ollinger & Taha, 2015). Therefore, this finding seems to suggest that the South African standard for the importation of MRPM concerning *Salmonella* species is lower than the international standard (DAFF, 2011). The odds

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of MRPM consignment to be contaminated with *Salmonella* species significantly differed based on the country. This is not surprising as varying prevalences of *Salmonella* have been reported in poultry products depending on the country and the area within the country (EFSA, 2008; Khan et al., 2018; Thames & Sukumaran, 2020). This could be attributed to differences in legislation, hygiene practices, biosecurity, disease control, and farming practices (Campos et al., 2018; Akramzadeh et al., 2020).

Therefore, country and region specific risk evaluation models must be developed to quantify and mitigate the risk of importing *Salmonella*-contaminated consignments (WHO, 2002).

Mechanically recovered poultry meat consignments imported in 2016 had an increased risk for *Salmonella* contamination compared to those in 2017. However, over the two years, there was a monthly increase in the number of consignments contaminated with *Salmonella* species. The reason for temporal changes is not clear in the literature. However, it is possible this could be due to changes in the enforcement at the port of entry and improved process control in exporting countries (Rose et al., 2002). Nonetheless, more research is needed to investigate the increasing trend in the number of contaminated consignments.

There was seasonal variation in the odds of MRPM consignments contaminated with *Salmonella* species. Similarly, other studies have reported seasonality in the proportion of poultry products contaminated with *Salmonella* species (Zdragas et al., 2012; Regalado-Pineda et al., 2020). Therefore, the risk-adjusted model for mitigation of the likelihood of the importation of MRPM consignment contaminated with *Salmonella* must also be taken into consideration as well as the seasonal variation in the levels of contamination.

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An importer was also a significant predictor of the likelihood of a consignment to be contaminated with *Salmonella* species. This is to be expected since importers have a certain preference for the country from which they want to import. Considering, importers should be engaged as part of the risk communication strategy for *Salmonella* control to ensure that they import from locations where the risk is negligible.

3.6 Limitations of the study

The authors did not have control over the variables collected and the process of collecting the data. In addition, the study focuses on the MRPM imported through a single port of entry. Therefore, the results of this study may not reflect situations at all ports of entry in South Africa. In view of this, more studies are needed to investigate the microbial contamination of MRPM imported through other ports of entry into South Africa. Nonetheless, the findings of the study will contribute significantly to our understanding of the risk of importing MRPM contaminated with *Salmonella* into South Africa.

3.7 Conclusion

Salmonella species were isolated in MRPM consignment imported into South Africa over the study period. The risk of importing a *Salmonella*-contaminated consignment was significantly associated with the country, the importer, and the time of importation. The procedure for handling *Salmonella*-contaminated MRPM must be amended in line with the Meat Safety Act 40 of 2000 and the international best practice. A QRA model must be developed to quantify the likelihood of importation of a contaminated consignment and identify potential drivers.

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Chapter 4

Quantitative risk assessment of *Salmonella* contaminated mechanically recovered poultry meat imported into South Africa.

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4.1 Abstract

Salmonella has been identified as a pathogen of public health concern as it has been linked with foodborne disease outbreaks globally. In this study, a quantitative risk assessment approach was used to quantify the likelihood of importing mechanically recovered poultry meat contaminated with *Salmonella* into South Africa. The approach has been used before to evaluate the risk associated with the import or export of animal commodities.

A quantitative risk analysis model was developed in Microsoft Excel. The Monte Carlo simulation was run at 10,000 iterations using a commercial software. Sensitivity analysis was performed to identify which factors had a significant impact on the overall likelihood of importation of contaminated recovered poultry meat.

The overall likelihood of importing mechanically recovered poultry meat contaminated with *Salmonella* into South Africa was calculated as 5.88 x10^{-4} (5th=

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5.886x 10^{-5}--95^{th}=5.73x10^{-3}). The prevalence of *Salmonella* in the exporting country (ρ=1.00), the sensitivity of the culture (ρ=0.05) and PCR (ρ=0.05) test, the within consignment prevalence (ρ=0.02), state decision (ρ=0.03) were positively correlated with the probability of importing a contaminated mechanically recovered poultry meat consignment. While the number of consignments imported into the country was negatively correlated (ρ=-0.01) with the probability of importing a contaminated consignment.

A quantitative risk assessment model shows that there is a likelihood of importing mechanically recovered poultry meat consignment contaminated with *Salmonella* species. Therefore, caution must be exercised, and continuous vigilance must be implemented, as a previous study on the prevalence of *Salmonella* species in mechanically recovered poultry meat consignment has reported the presence of zoonotic *Salmonella* serotypes.

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4.2 Introduction

Mechanically recovered poultry meat is a nutritious and affordable source of protein (Hecer & Sözen, 2011; Pereira et al., 2011; Chauhan et al., 2017; Lombard & Bahta, 2018). Of recent, there has been an increase in the demand for MRPM, consequently, the importation of MRPM into South Africa has also increased (SAPA, 2016; Kapuya, 2017; Makgopa & Bonsu, 2020). The increase in importation was due to a decrease in chicken prices in South Africa thus resulting to a decrease in production (Kapuya, 2017). It is estimated that 190 000 to 200 000 tonnes of MRPM imported into South Africa between 2016 and 2017 mainly from Brazil (BFAP, 2019). It is important to note that the importation of MRPM into South Africa has a zero tariff, ensuring affordability for households with low income (Kapuya, 2017).

Despite evidence of the significant contribution of trade in animal and animal products to the spread of infectious diseases with a negative impact on the health of animals and humans (Evans & Leighton, 2014; Lekshmi et al., 2017), there are limited published studies on *Salmonella* species in imported meat products to South Africa. Elsewhere, MRPM has been reported as a source of pathogenic organisms including *Salmonella* species (Akpabio, 2015; Ali & Sultana, 2016; Das et al., 2017). In addition, food-borne disease outbreaks have been reported among immunocompromised persons associated with the consumption of contaminated MRPM (van Nierop et al., 2005; Gordon, 2008; Sigaúque et al., 2009; Khan et al., 2010; Kidanemariam et al., 2010; Majowicz et al., 2010; Akpabio, 2015; Magwedere et al., 2015; Ali & Sultana, 2016; Das et al., 2017; Muvhali et al., 2017).

In view of the reported outbreaks, a holistic approach is required to prevent and control human *Salmonella* infections (FAO & WTO, 2017). One of such approach is

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estimating the likelihood of *Salmonella* contamination or infection in meat products using the QRA approach (CAC, 2007). The QRA is a preferred approach as it takes a mathematical approach to estimate the likelihood and severity of a risk (Lammerding & Fazil, 2000; Dong et al., 2015). This technique has become a method of choice as it can characterize the variability and uncertainty of a risk (Schlundt, 2000; Lammerding & Fazil, 2000; Zwietering & van Gerwen, 2000; FAO & WHO, 2003; Lammerding, 2006; EFSA, 2008; Mead et al., 2010; Oscar, 2011; OIE, 2021). It considers various mitigation steps through the value chain to identify to most likely entry for a hazard (Cassin et al., 1998).

Import QRA is divided into entry assessment, exposure assessment, consequence assessment, and risk estimation (Zwietering & van Gerwen, 2000; Kelly et al., 2003; Oscar, 2011; Kalaba et al., 2017; OIE, 2021). Entry assessment describes the pathway in which *Salmonella* can be introduced into an importing country and the likelihood of this occurring. The steps also look at how the likelihood of entry changes based on biological, country, and consignment factors. In terms of *Salmonella* these may include testing at the port of entry, documentation, inspection, and compliance reports (DAFF, 2008; Kuznetsova, 2016; OIE, 2021). Exposure assessment describes pathways in which an imported risk product like MRPM might expose *Salmonella* to humans and animals. Consequence assessment involves identifying consequences after exposure which could be either health importance, economic or political (USDA, 2003; Majowicz et al., 2010; Cosby et al., 2015; Peeler, Reese & Thrush, 2015; Sergeant, 2015; Hao et al., 2016; Suijkerbuijk et al., 2016; Muvhali et al., 2017; OIE, 2021). Lastly, risk estimation integrates entry, exposure, and consequence assessments to estimate the risk. In a QRA model, the risk estimate may include the probability distributions, confidence intervals, variance of model inputs and analysis,

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and correlation between model inputs (Cassin et al., 1998; Lammerding & Fazil, 2000; Kuznetsova, 2016; OIE, 2021).

In this study, a QRA was used to estimate the likelihood of importing MRPM contaminated with *Salmonella* into South Africa. In addition, sensitivity analysis was used to describe the impact of the events on the probability of importing a *Salmonella*-contaminated consignment. The study hypothesizes that the likelihood of importing MRPM contaminated with *Salmonella* into South Africa is high and that the current mitigation steps are not adequate to reduce the likelihood of importing a contaminated consignment. The results of this study will contribute to the scientific basis for the control of *Salmonella* originating from imported MRPM.

4.3 Materials and methods

4.3.1 Ethics approval

This study was approved by the DALRRD and Animal Ethics Committee, and the Research Ethics Committee of the University of Pretoria (REC 182-19).

4.3.2 Study area

A secondary dataset of MRPM consignment imported through a port of entry into South Africa was used for this study. Furthermore, the study used data from peer-reviewed published studies between 2016 and 2017.

4.3.3 Process of importation of mechanically recovered poultry meat into South Africa

All importers are required to apply for an import permit with the DALRRD. Then the department evaluates the risk of importing a commodity into the country based on the guidelines of the Animal Diseases Act 35 of 1984 and the Meat Safety Act 40 of 2000. Similarly, a country may express interest in exporting to South Africa, a

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questionnaire would then be sent to that country to begin the process of information gathering, followed by an inspection visit to address the animal disease status, veterinary control, and surveillance programs, and meat safety aspects (DAFF, 2008). Once the risk has been determined to be negligible, a VIP with a sanitary certificate is issued to facilitate the process of importation. In addition, approved facilities are then listed as establishments to import from. The VIP is usually valid for six months and can only be used once for one consignment. During importation, the veterinary health certificate from the veterinary authority of the exporting country must accompany the consignment (DAFF, 2008).

4.3.4 Hazard identification

Hazard identification is the preliminary step required in a QRA for a substance considered to have public health or economic significance (Cassin et al., 1998; Lammerding & Fazil, 2000; WHO, 2008). In this study, *Salmonella* a bacterial pathogen known to cause diseases in humans and animals was identified as a hazard. The bacterium has been reported in several studies in South Africa. However, limited studies have demonstrated the presence of the organism in meat and meat products including MRPM (Peeler, Reese & Thrush, 2015; Sergeant, 2015).

4.3.5 Risk Assessment

This is a step subdivided into entry assessment, exposure assessment, and consequence assessment. Entry assessment is a process in which a hazard is introduced into an importing country and the likelihood of this occurring. The likelihood of entry may be influenced by biological, country, and commodity factors. Exposure assessment described ways by which an animal or human of the importing country might be exposed and the likelihood of this to occur. Lastly, consequence assessment

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is a relationship between a specific exposure and the adverse consequence of that exposure (Sergeant, 2015; OIE, 2021).

4.3.6 Quantitative Risk Assessment Modelling Approach

4.3.6.1 Scenario tree

A scenario tree outlining the questions that were asked during the development of the risk pathway model is described in **(Figure 4.1)**. It is composed of nodes (P1-P6), each node defined a step in the pathway, and questions asked related to failure to mitigate the risk identified. The scenario tree model follows the most common importation process of MRPM from exporting countries to South Africa **(Figure 4.1)**.

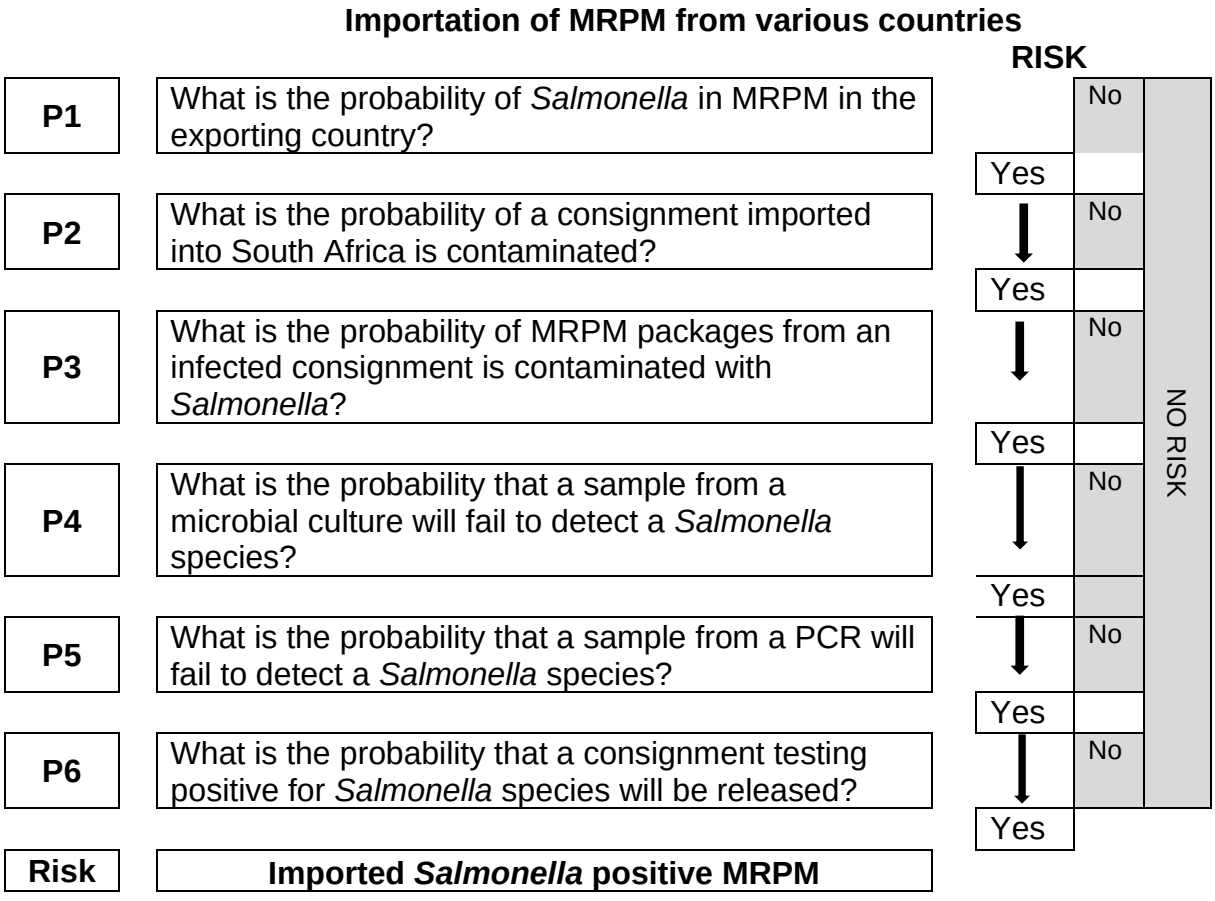

Figure 4.1: Developing the scenario tree for likelihood of MRPM to be contaminated with *Salmonella*.

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4.3.6.2 Assumptions of the model

The model assumes that all mitigation steps have been followed by the exporting country before an MRPM is exported to South Africa.

4.3.7 Data source

The study uses the dataset of MRPM imported into South Africa between 2016 to 2017, which was supplied by DALRRD. The study also relied on relevant published studies over the same study period.

4.3.8 Model design

A quantitative risk assessment model (QRAM) for the likelihood of importing an MRPM consignments contaminated with *Salmonella* was constructed in Microsoft Excel (Microsoft Redmond. WA). The Monte Carlo simulation was performed using a commercial software @Risk (version 7.6, Palisade, Newfield, NY) at 10,000 iterations.

4.3.9 Evidence gathering and parameter estimation

The probability of an exported consignment to South Africa being contaminated (P1)

The probability of an exported consignment being contaminated with *Salmonella* was calculated by using data from published studies. Data was collected from eight studies selected based on a systematic review. The data was then fitted using palisade @Risk to determine the best fit model (Vose, 2008). In the final analysis, the risklevy fit the data better, therefore, the probability that a country exporting a contaminated consignment was calculated as 0.09 (0.00751 -1.00).

4.3.10 A systematic literature review for P1

A systematic literature review was conducted to gather data on the prevalence of *Salmonella* contamination in MRPM from countries exporting to South Africa.

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Keywords and synonyms/proxy words used in various databases are shown in **(Table 4.1)**.

Table 4.1: Table of keywords and synonyms for search engines.

Keywords	Synonyms/Proxy words
Prevalence	Proportion
	Probability
	Occurrence
	Frequency
Salmonella	Salmonellosis
Mechanically Recovered Meat	Mechanically Separated Meat
	Mechanically Deboned Meat
Poultry	Chicken
	Avian

4.3.10.1 Eligibility criteria

The data was collected from published peer-reviewed primary research studies, written in the English, published between 2016 and 2017. The study focuses on countries exporting MRPM to South Africa. Details of the electronic databases and search terms used to identify studies to be included are provided in **(Table 4.2)**. Boolean operators were used in all searches. All eligible studies and their associated documents were captured using Mendeley reference manager. From each study, the following information was extracted, authors, year of publication, country, number of samples tested and number of positive cases.

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Table 4.2: Search terms and databases used in the systematic review in the exporting countries.

Publications	Search terms
Google scholar	"Prevalence OR "Proportions" OR "Occurrence" AND "*Salmonella*" AND "Poultry" OR "Chickens" "*Salmonella*" AND "Prevalence" OR "Proportions" AND "Mechanically Deboned Meat" AND "Poultry" AND "European countries" OR "Brazil" OR "Netherlands" OR "Argentina" OR "Canada" OR "Thailand" "Occurrence" AND "*Salmonella*" OR "Salmonellosis" AND "Mechanically Separated Meat" AND "European countries" OR "Brazil" OR "Netherlands" OR "Argentina" OR "Canada" OR "Thailand"
Web of Science	"Prevalence OR "Proportions" OR "Occurrence" AND "*Salmonella*" AND "Poultry" "*Salmonella*" OR "Salmonellosis" AND "Prevalence" OR "Proportion" AND "Chickens" OR "Poultry" AND "European countries" AND "Brazil" AND "Netherlands" AND "Argentina" AND "Canada" AND "Thailand"
Science Direct	"Prevalence OR "Proportions" OR "Occurrence" AND "*Salmonella*" AND "Poultry" OR "Chickens" "*Salmonella*" OR "Salmonellosis" AND "Prevalence" OR "Proportion" AND "Chickens" OR "Poultry" AND "European countries" AND "Brazil" AND "Netherlands" AND "Argentina" AND "Canada" AND "Thailand" "*Salmonella*" AND "Chickens" OR "Poultry" AND "European countries" AND "Brazil" AND "Netherlands" AND "Argentina" AND "Canada" AND "Thailand" "*Salmonella*" OR "Salmonellosis" AND "Prevalence" OR "Proportion" AND "Mechanically Separated Meat" AND "Chickens" OR "Poultry" AND "European countries" AND "Brazil" AND "Netherlands" AND "Argentina" AND "Canada" AND "Thailand"
PubMed	"Prevalence OR "Proportions" OR "Occurrence" AND "*Salmonella*" AND "Poultry"
Scopus	"Prevalence OR "Proportions" OR "Occurrence" AND "*Salmonella*" AND "Poultry" OR "Chickens"

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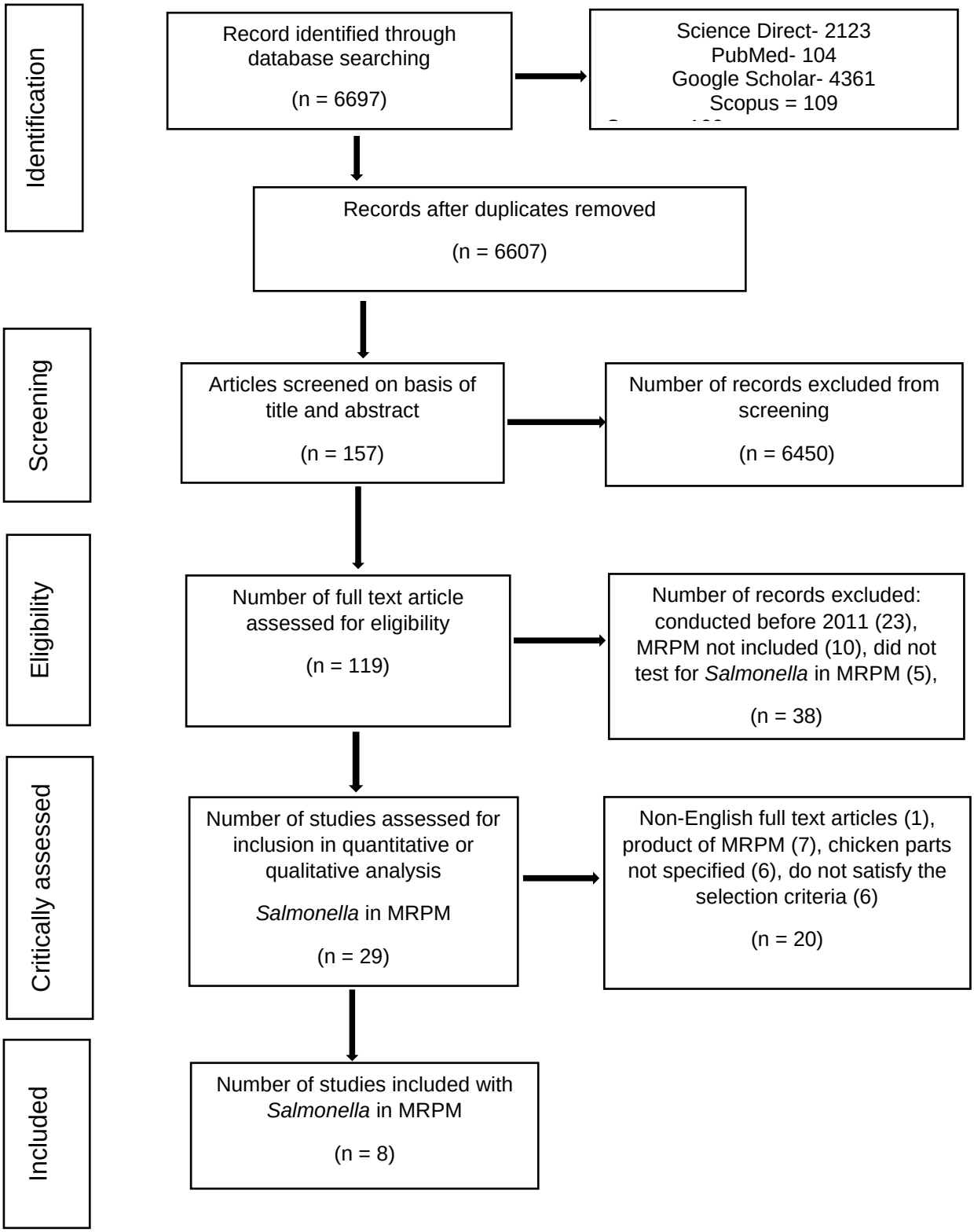

Figure 4.2: PRISMA diagram of the systemic review of MRPM contaminated with *Salmonella*.

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Based on the systematic review, the highest (100%) prevalence of *Salmonella* in MRPM in the USA was reported in a study by (Taylor et al., 2015), followed by 28% reported by (Alali et al., 2016) and 12% by (Hutchinson, Wheeler & Mohle-Boetani, 2018). In the European countries, 13% prevalence was reported in a study by (Martynenko & Rula, 2020), 6% by (Kalaba *et al.*, 2017) followed by 5% (Tőzsér et al., 2019), and 4% by (Modzelewska-Kapituła & Maj-Sobotka, 2014). While a Brazilian study reported a 1% prevalence in MRPM (Neves et al., 2018) **(Table 4.3)**.

Table 4.3: List of papers selected from systemic review to obtain prior distribution of *Salmonella* in MRPM.

Author	Reference	Country	No. of samples	No. of positive	Proportion
Kalaba et al.	*Earth Environ. Sci.*, 2017, 85	Europe	265	15	0.06
Hutchinson et al.	*Epidemiology and Infection*, 2018, 146: 430-436	USA	341	42	0.12
Neves et al.	*Acta Scientiae Veterinariae*, 2018, 44: 1418	Brazil	162	1	0.01
Modzelewska-Kapituła & Maj-sobotka	*Food Safety*, 2014, 34:126–131	Poland	110	4	0.04
Martynenko & Rula	*Veterinary Medicine, Biotechnology and Biosafety*, 2020, 6:2411-3174	Ukraine	68	9	0.13
Taylor et al.	*Foodborne Pathogens and Disease*, 2015, 12: 950-952	USA	23	23	1.00
Alali et al.	*Food Control*, 2016, 66:250-255	USA	180	50	0.28
Tőzsér et al.	*Acta Alimentaria*, 2019, 48:488-494	Hungary	110	5	0.05

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Table 4.4: Variables and probability distributions used in the model for likelihood of importing *Salmonella*-contaminated MRPM.

Input	Unit/scale	Definition	Parameter estimate	References
N	Number of importations	Count	Riskuniform (min, max)	Unpublished data
P1	Freezing and transportation	Prevalence	Risklevy (μ, c)	Table 3
P2	Importation of contaminated consignments	Prevalence	Beta (α_1, α_2)	Woube et al., 2015
P3	Package positive	Prevalence	Beta (α_1, α_2)	Vose., 2008
P4	Culture sensitivity	Sensitivity	Riskuniform (min, max)	Gaillot et al., 1999
P5	PCR Sensitivity	Sensitivity	Riskuniform (min, max)	Yoshida et al., 2016
P6	Releasing positive consignment	Prevalence	Riskuniform (min, max)	Woube et al., 2015
Likelihood		P1*P2*P3*P4*P5*P6		

Number of consignments into the country yearly (N)

The number of consignments imported were calculated using risk uniform (2746,3119) were 2746 was number imported in 2016 and 3119 is number imported in 2017. Therefore, the number of consignments imported between 2016 and 2017 was calculated to be 2933**.**

The probability of Salmonella species in MRPM surviving freezing and transportation (P1)

Mechanically recovered poultry meat are frozen, however, a failure to maintain the cold chain can occur. Concerning *Salmonella* in MRPM, refrigeration preserves food by slowing down the growth and reproduction of microorganisms as well as the

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action of enzymes. Studies have shown excessive bacterial growth occurs when temperature exceed 5.2˚C (Collineau et al., 2020; Oscar, 2017; Zhu et al., 2017). In this study, the assumption was that it's highly unlikely that the temperature during transporting exceeded 5.2˚C.

The probability of a contaminated consignment being imported into South Africa (P2)

Probability of an imported consignment being contaminated was assessed using a beta distribution with p=(r+1, n-r+1) where r=(P1) and n=number of consignments imported into the country. Therefore, the probability of an imported consignment being contaminated was calculated as (0.09+1, 2933-0.09+1) which is calculated to be $3.7x10^{-4}$.

The probability that a package sample from a contaminated consignment is contaminated with a Salmonella (P3)

Upon arrival at the port of entry in South Africa, each consignment of the MRPM is tested for the presence of microbiological hazards (DAFF, 2008). Firstly, MRPM consignments are checked for signs of thawing. A temperature of -18˚C is preferred, however, -10˚C or below is still acceptable. From each consignment, five samples are randomly selected from different packages. It is estimated that each consignment contains between 1632.6 packages (20kg) and 2448.9 (15kg) depending on the size of the package. Each sample taken must weigh at least 50 grams. After collection, samples are then placed in a cooler container and must reach the laboratory before the temperature rises above 4˚C.

The probability that a sample/package tests positive (within consignment prevalence) was assumed to follow a beta distribution (α_1=r+1, α_2=n-r+1) where r is

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the number of *Salmonella* positive packages, and n is the number of samples/ packages tested. The within consignment prevalence was calculated based on the laboratory data of consignments imported into South Africa between 2016 and 2017. Therefore, r=985 of the n=28665 samples submitted to the laboratory for microbial analysis tested positive for *Salmonella*. Therefore, the within consignment prevalence was calculated as 0.03. Since not all packages are tested, the probability of at least one of the five packages testing positive for *Salmonella* was estimated to be 0.15 (number of samples collected × within consignment prevalence) (Vose, 2008).

Probability that a microbial culture will fail to detect a Salmonella species (P4)

The probability that a culture test detects contaminated samples was calculated using riskuniform (minimum, maximum). Culture sensitivity used was based on the CHROMagar *Salmonella* (CAS) culture method which has a sensitivity of 80% to 100% respectively (Gaillot, Di Camillo, Berche, Courcol & Savage, 1999). Therefore, the following calculation was used, riskuniform (0.80, 1.00) with the probability estimated to 0.90. Therefore, the probability of a culture test to fail to detect one positive consignment was calculated as 1-*p*.

Probability that a sample from polymerase chain reaction will fail to detect a Salmonella species (P5)

The probability that the PCR test fails to detect a *Salmonella* positive sample was also calculated using riskuniform (minimum, maximum). The sensitivity for PCR was based on four methods; *Salmonella* genoserotyping array (SGSA), *Salmonella* serogenotyping assay (Salm SeroGen), check and trace *Salmonella* and xMAP *Salmonella* serotyping assay with a sensitivity of between 74% and 95% (Franklin et al., 2011; Yoshida et al.,, 2016; Yoshida et al., 2014). Therefore, the following

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calculation was used, riskuniform (0.74, 0.95) with the probability estimated at 0.85. Therefore, the probability that the PCR test fails to detect one positive consignment was calculated as 1-*p*.

Probability that a consignment testing positive for Salmonella species will be released (P6)

The South African SOP allows for importation of commodities provided that no more than three out of five samples test positive for *Salmonella* species with the exclusion of *Salmonella* Typhi*, Salmonella* Enteriditis, and *Salmonella* Typhimurium. The probability that a consignment testing positive for a *Salmonella* species and be released by a state veterinarian was assumed to follow a beta distribution (r+1; n-r+1), where r is the number of *Salmonella* positive consignments and n is the number of *Salmonella* samples that were released by the state veterinarian. Therefore, using beta (228+1, 632-228+1) the probability was estimated to be 0.36.

Probability that a consignment will test positive

The overall probability of an imported consignment testing positive for *Salmonella* was calculated as P1*P2*P3*P4*P5*P6 where P1 is the probability of *Salmonella* in MRPM in the exporting country, P2 is the probability of an imported consignment being contaminated, P3 is the probability that an MRPM package/sample from a contaminated consignment is contaminated, P4 probability of a culture test to fail to detect a contaminated sample/package, P5 probability of a PCR test failing to detect a contaminated consignment/package and P6 is the probability of a *Salmonella* positive consignment being released by the state veterinarian. In this study, the overall probability that a consignment will test positive is 5.88 $x10^{-4}$ (5^{th}= 5.886x 10^{-5}--95^{th}=5.73x10^{-3}).

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4.3.11 Modelling predictor relationship

Spearman's ranked correlation coefficient test was used to assess the strength of relationship between different probabilities/stages and the overall probability of importing a *Salmonella*-contaminated consignment. The following probabilities/stages: country (prevalence of *Salmonella* in the exporting countries), package (within consignment prevalence), culture (sensitivity of the test), PCR (sensitivity of the test), state decision (releasing a consignment), consignments (number of consignments imported into the country) were assessed against the overall probabilities of a consignment to be contaminated with *Salmonella*.

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4.4 Results

The overall likelihood of importing *Salmonella*-contaminated MRPM into South Africa was calculated as a medium of 5.88 x10^{-4} (5^{th}: 5.886x 10^{-5}--95^{th}: 5.73x10^{-3}) **(Figure 4.3) (Table 4.5)**.

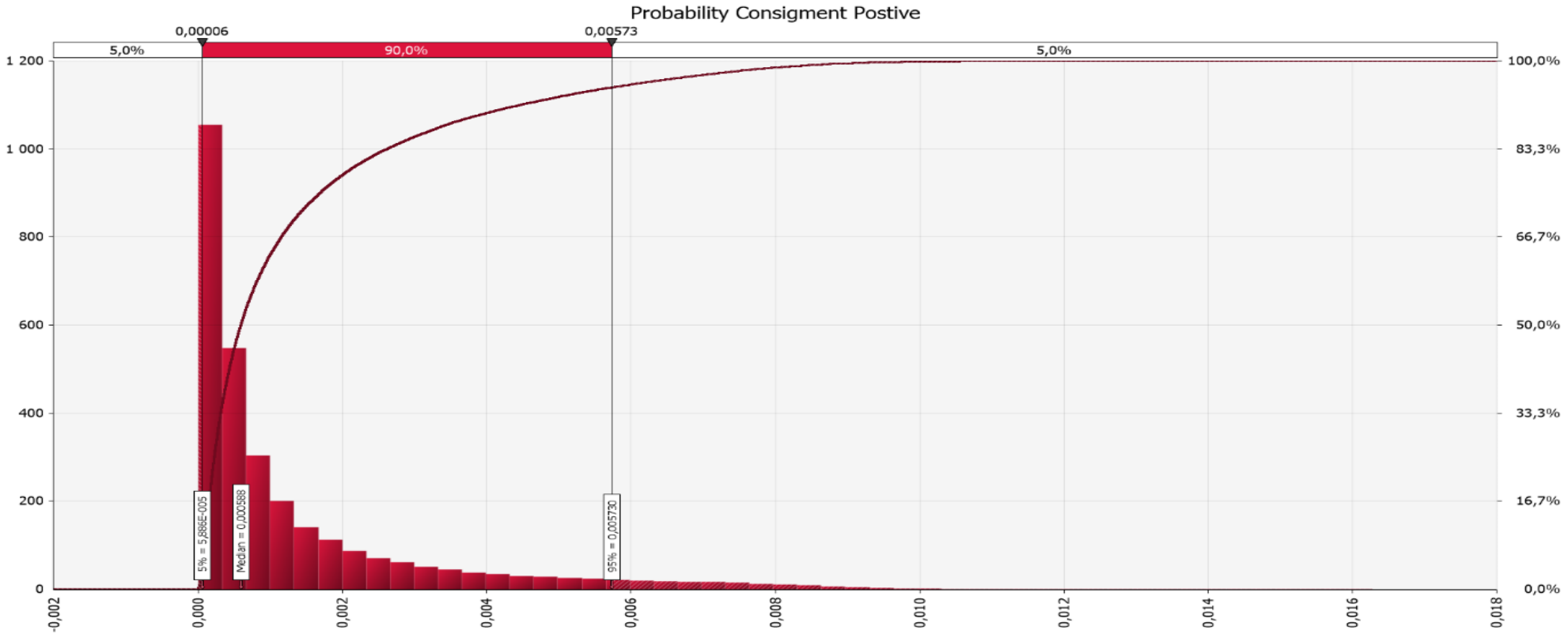

Figure 4. 3: Probability distribution showing likelihood of importing MRPM infected with *Salmonella* into South Africa.

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Table 4.5: Simulation results for estimations related to the likelihood of importation of *Salmonella* in MRPM.

	Minimum	5th Percentile	Mean	95th Percentile	Maximum
N	2746	276.4	2932.50	3100.3	3119
P1	0.09[a]	0.00751	0.05	1.00	-
P2	$2.864X10^{-008}$	$1.74x10^{-5}$	$3.414X10^{-3}$	$1.029X10^{-2}$	$3.03x10^{-2}$
P3	0.00	0,031	0.033	0.0343	1.00
P4	0.80	0.81	0.90	0.99	1.00
P5	0.74	0.75	0.85	0.94	0.95
P6	0.00	0.33	0.36	0.39	1.00

[a]Median= risklevy does not produce the mean

The results of the spearman's correlation analysis indicate that the likelihood of importing a *Salmonella*-contaminated MRPM consignments was strongly associated with the initial prevalence of *Salmonella* in MRPM in the exporting countries (1.00). Weak to no association was observed between the sensitivity of culture (0.05) or PCR (0.05) test to detect a contaminated sample, the decision of the state veterinarian to release the consignment (0.03), the within consignment prevalence of *Salmonella* (0.02) and the probability of importing a *Salmonella*-contaminated consignment. Similarly, the number of consignments imported had a negative association with the likelihood of importing a *Salmonella*-contaminated consignment **(Figure 4.4)**.

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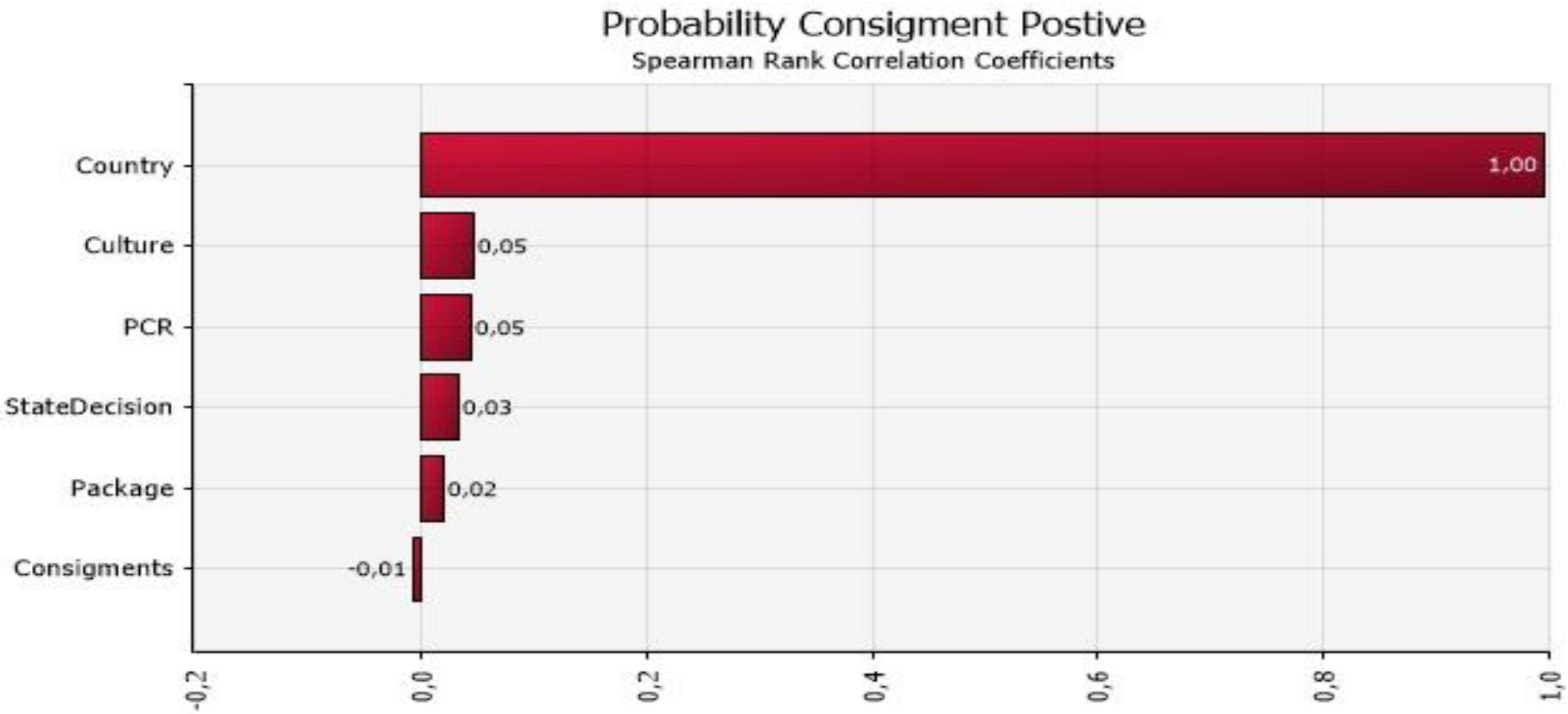

Figure 4. 4: Correlation coefficient analysis for the likelihood of importing MRPM contaminated with *Salmonella* into South Africa.

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4.5 Discussion

Salmonella species have been reported as a cause of foodborne disease globally (Lekshmi et al., 2017) with *Salmonella* Typhimurium being common among patients with human immunodeficiency virus (HIV) (Gordon, 2008). Although there is evidence that imported meat products are likely to be a source of *Salmonella*, the probability of an MRPM consignment imported being contaminated has not been investigated (van Nierop et al., 2005; Magwedere et al., 2015). Therefore, this study investigated the likelihood of importing MRPM contaminated with *Salmonella* and identified associations of contamination with each step mitigation.

In this study, the likelihood of importing MRPM contaminated with *Salmonella* into South Africa was present (OIE, 2010). It is important to note that there is no proper guidance on the acceptable level of risk (MacDiarmid & Pharo, 2003; Peeler, Reese & Thrush, 2015). Notwithstanding, in chapter 3 of the dissertation on the prevalence of *Salmonella* in MRPM, *Salmonella* serotypes known to cause foodborne illnesses including *Salmonella* Heidelberg and *Salmonella* Schwarzengrund have been reported in South Africa. Moreso, these *Salmonella* serotypes have been reported in human outbreaks associated with consumption of MRPM (Hoffmann et al., 2013; Alali et al., 2016; Neves et al., 2018; Knodler & Elfenbein, 2019; Motladiile et al., 2019; Ndobeni, Magwedere & Qekwana 2022). Of concern is that the South African SOP allows importation of *Salmonella*-contaminated commodities excluding *Salmonella* Typhi, *Salmonella* Enteriditis, and *Salmonella* Typhimurium (DAFF, 2011). Therefore, consideration must be made to align this SOP with the regulations of Meat Safety Act 40 of 2000 which requires identification, prevention, elimination, or reduction of a pathogen to an acceptable level (DAFF, 2000, 2006). Following these practices this

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will align the South African SOP with the best international practices on *Salmonella* prevention (EU, 2014; Jansen et al., 2018)

The prevalence of *Salmonella* in the exporting countries was positively correlated with the overall likelihood of importing *Salmonella* species contaminated consignment. This positive correlation is consistent with studies that have shown the important role played by the infectious disease status of the country on the likelihood of importing a pathogenic organism. This has necessitated the importing country to conduct inspection visits and assess disease control and surveillance programs to ensure that adequate risk mitigation measures are efficient and effective (Habtemariam, 2005; DAFF, 2008). In view of the above, both qualitative and quantitative risk assessments must be undertaken systemically and regularly to ensure correct measures are in place in exporting countries. In addition, arbitrary acceptable level of risk must be clearly defined and empirically set to ensure consensus on the acceptable risk (MacDiarmid & Pharo, 2003; Peeler, Reese & Thrush, 2015).

Detection of *Salmonella* bacteria is mainly based on culture and PCR (Gelaw et al., 2018; Kolo et al., 2019). Although culture is the gold standard, inexpensive, and easily accessible, it can take up to seven days to receive results (Zhao et al., 2014; Foddai & Grant, 2020). In addition, the culture test has lower sensitivity when compared to PCR (Ellis et al., 2019). For example, Rychlík et al. (1999) and Melendez et al., (2010) reported culture sensitivity of 50%, respectively (Melendez et al, 2010). Polymerase chain reaction is a robust and a rapid molecular method used to detect foodborne pathogens (Zhao et al., 2014; Afzal et al., 2015). PCR serotyping provides standardization and reproducibility results (Salazar et al., 2015) and a sensitivity of 92% has been reported (Rychlík et al. 1999).

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In the current study, the sensitivities of culture and PCR tests were positively correlated with the likelihood of importing a positive consignment into the country. However, the value was closer to zero suggesting that the association is weak. Nonetheless, identification of biological hazards using culture and PCR diagnostic tests continue to be an important step in mitigating risk associated with importation of meat products (Gelaw et al., 2018).

The within consignment prevalence and decision of the state veterinarian to release were weakly associated with the overall likelihood of *Salmonella* in MRPM. Decision of the state veterinarian to release the consignment requires that no *Salmonella* Typhi, *Salmonella* Entereditis, and *Salmonella* Typhimurium is detected during PCR testing or no more than three samples should test positive for other *Salmonella* species (DAFF, 2011). Therefore, the within consignment prevalence is important for the state veterinarian deciding on suitability of the entire consignment for acceptance into the country. It is important to note that this standard is lower than zero samples testing positive for *Salmonella* in the EU (EU, 2014; Jansen et al., 2016) and five out of 51 samples in the US (Ollinger & Taha, 2015). This suggests that South African standards on importation are inadequate compared to international standards and other trading partners (DAFF, 2011).

4.6 Limitations of the study

The study focused on the MRPM imported through a single port of entry in South Africa. Therefore, the results of this study may not reflect situations at all ports of entry in South Africa. In view of these, more studies covering all the ports of entry, and more carefully selected samples, representative of all the imported meat products are needed to investigate the likelihood of importing MRPM contaminated with *Salmonella* at other ports of entry into South Africa. Nonetheless, the findings of this

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study form the baseline for future studies on QRA associated with import of meat and meat products into South Africa.

4.7 Conclusion

The likelihood of importing a *Salmonella*-contaminated consignment in the current study was positively correlated with the prevalence of *Salmonella* in the exporting country, within consignment prevalence, sensitivity of culture and PCR test, state veterinary decision to accept or reject a consignment, and the number of consignments imported. However, it must be noted that in the previous study zoonotic *Salmonella* serotypes were isolated and this should be concerning. Therefore, more risk based improvements must be undertaken to align the real time foodborne and zoonotic risk evaluation through importation with emerging *Salmonella* trends, and on the integration of QRA into the port-level risk assessment in South Africa.

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Chapter 5

5.1 Summary, conclusions, and recommendations

This study has evaluated the import risk of MRPM consignment contaminated with *Salmonella* using QRA approach and confirmed some significant findings. The current risk is negligible yet present. The prevalence of *Salmonella* in MRPM imported into SA is similar to what was obtained in US and Belgium but lower than those from other studies in US and Estonia. The differences may be due to differences in legislation, hygiene and farming practices, biosecurity, and disease control. We observed seasonal variation in the odds of MRPM consignments testing positive for *Salmonella* species, and the importer was a significant predictor of the likelihood of a consignment testing positive for *Salmonella* species. This could be due to the fact importers have a certain preference for the country of export, and this should guide policy decisions.

In view of the above, the recommendations are that a QRA is done to evaluate the current risk mitigation strategies if needed to make recommendations towards effective monitoring and surveillance. In addition, the South African important regulations on MRPM must be reviewed to align with the international standard. Country and region specific risk evaluation models must be developed as the odds of importing *Salmonella* positive MRPM consignment differed based on the country of origin. Since an importer was an important factor in the likelihood of importing a *Salmonella* positive consignment, an effective risk communication strategy must incorporate the characteristics of the importer.

In the second manuscript, a QRA was developed to quantify the likelihood of importing a consignment contaminated with *Salmonella* and this was present.

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However, the results of the first objective identified the presence of zoonotic *Salmonella* serotypes of public health importance to South Africa. Therefore, there is a need to implement targeted strategies to reduce the risk further. The results of the sensitivity analysis show that compared to others the initial prevalence in the exporting countries was significantly associated with the increased likelihood of a consignment testing positive for *Salmonella*. This further emphasises the need for import certification to ensure that the *Salmonella* disease status of the exporting country is negligible.

Sensitivity of culture and PCR tests, the within consignment prevalence, and state decision to release the consignment also had an influence on the likelihood of a consignment testing positive for *Salmonella*. However, the impact of these measures was not that significant compared to that of the initial prevalence in the exporting country. Notwithstanding the low impact, the decision to allow the importation of MRPM consignments contaminated with other *Salmonella* serotypes should be reviewed to align with the international standards. Moreover, EU regulations require that no MPRM package test positive for *Salmonella*.

5.2 Conclusion

Salmonella species that have been known to cause zoonotic disease were isolated in MRPM consignment imported into South Africa in this study. Country of origin, the importer, and the time of importation were factors to consider when importing MRPM consignment into South Africa. The likelihood of importing a *Salmonella*-contaminated consignment was present and positively associated with the prevalence of *Salmonella* in the exporting country, the within consignment prevalence, the sensitivity of culture and PCR, state decision to release the consignment, and

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negatively associated with the number of consignments imported. Since South Africa imports all its MRPM, effective monitoring and surveillance programmes must be implemented to prevent potential future outbreaks in humans and animals.

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