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Prevalence and correlates of Campylobacter and Salmonella species in small-scale broiler poultry, Gauteng, South Africa, 2020-2021

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Prevalence and correlates of *Campylobacter* and *Salmonella* species in small-scale broiler poultry, Gauteng, South Africa, 2020-2021

Shira Rebeka Amar

A Research Report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science (Epidemiology).

Johannesburg, June 2022

DECLARATION

I, Shira Rebeka Amar, declare that this research report is my own original work. Where another person's work has been used (either from a printed source, internet or any other source), it has been acknowledged and referenced in accordance with the guidelines of the Wits School of Public Health, University of the Witwatersrand. I have not used work previously produced by another student or any other person to hand in as my own. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work. This research has not been submitted previously for any other degree or examination at any other institution.

Shira Rebeka Amar

Signed: 

Date: 22/06/2022

DEDICATION

In loving memory of my mother,

Ruth Lapinsky Amar

(1960-2021)

“צָמְאָה לְךָ נַפְשִׁי“

Psalm 63:2

ABSTRACT

Background

Campylobacter spp. and non-typhoidal *Salmonella enterica* (NT *S. enterica*) serovars are commonly isolated zoonotic bacteria associated with foodborne disease in humans. Colonisation of broilers with these pathogens compromises food safety for consumers of poultry products. Prevalence estimates of *Campylobacter* spp. and NT *S. enterica* serovars in local small-scale broiler production systems are sparse. Furthermore, biosecurity measures practiced in these production systems are not documented. This study aimed to estimate the prevalence of *Campylobacter* spp. and NT *S. enterica* serovars among small-scale broilers and examine the correlation of biosecurity practices with *Campylobacter* spp. positivity in Gauteng Province, South Africa.

Methods

Secondary cross-sectional data from a survey of 30 small-scale broiler farms was analysed. Farmers completed a farm management and biosecurity questionnaire. Cloacal swabs from 900 broilers were cultured using standard methods for *Campylobacter* spp. and *Salmonella*. Isolates were identified using a real-time polymerase chain reaction test. Farm and bird-level prevalence was calculated. Biosecurity correlates of *Campylobacter* spp. were identified using univariable and multivariable logistic regression while accounting for clustering of broilers by farm. Potential confounders such as season, antibiotic use and age at slaughter were adjusted for.

Results

NT *S. enterica* serovars were not isolated (0% (0/900), 95% CI (0.0– 4.0%)). In contrast, *Campylobacter* spp. were detected in 49.8% of broilers (448/900, 95% CI (46.5–53.1%)) with an overall farm prevalence of 90% (27/30, 95% CI (73.4–97.9%)). *Campylobacter coli* was dominant (65.4%, 316/483) over *C. jejuni* (34.6%, 167/483). Among sampled broiler farms, 53.3% (16/30) applied rodent control methods and 56.7% (17/30) used soap and disinfectant for poultry house hygiene. Exposures correlated to *Campylobacter* spp. positivity included open-walled naturally ventilated houses compared to free-range housing (OR= 3.82 95% CI (2.09 – 6.97)), drinking water treatment (OR=86.81 95% CI (4.56 – 1651.50)) and unsealed feed bags or feed spillage (OR= 12.48, 95% CI (2.88 – 54.12)). Conversely, the summer season (OR=0.06, 95% CI (0.02–0.20)) was protective.

Conclusion

Campylobacter spp. were highly prevalent in sampled broilers while no NT *S. enterica* serovars were isolated. Appropriate drinking water treatment and correct feed management of high-quality feed and improved hygiene of poultry houses emerged as important biosecurity and farm management practices that may reduce the odds of *Campylobacter* spp. positivity in small-scale production systems. These findings may be used to improve the health of broilers produced in small-scale systems and promote food safety for consumers in this market sector.

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NOMENCLATURE

All-in-all-out system	A strategy used in animal rearing to minimise spread of infectious diseases. In this system, all animals are emptied from an area/premises and the area is cleaned and disinfected without any animals inside, before a new group of animals is introduced. Only animals of the same age group are reared at one time.
Antimicrobial resistance	The ability of a microbial pathogen to survive exposure to an antimicrobial agent that previously was able to destroy and control the microbe. Antimicrobial resistance arises through genetic mutations and transfer of genes.
Biosecurity	A set of management and physical measures that aim to reduce the risk of introduction, establishment and spread of animal diseases, infections or infestations, to, from and within an animal population.
Broiler	Chicken raised for the purpose of meat production.
Caecum / caeca	A pouch within the digestive tract: the blind-ending tube connected to large intestine in digestive tract of poultry.
Carcass rinse fluids	Fluids sampled after rinsing an animal carcass with water.
Cloaca	A common cavity at the end of the digestive tract for the release of excretory and reproductive products in birds and reptiles.
Colonise/ colonisation	The presence of a microorganism on or in a host, with growth and multiplication of the organism, but without interaction between host and organism.
Commensal/ commensalism	An organism (such as a bacteria) in a biological relationship that benefits from the interaction while the other organism is not harmed nor receives any benefit.
Contaminate/ contamination	The presence of a microorganism on a body surface or an inanimate object.
Flock	A group of animals of one kind (referring to birds or sheep).
Litter	A mixture of material used for bedding in a poultry house (e.g., sunflower husks, wood shavings, spilled feed, and feathers/excretions from the poultry that were housed.)

Partial depopulation	Early removal of a portion of birds from a poultry house while leaving some birds remaining in the house.
Poultry	All domesticated birds, including backyard poultry, used for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds, as well as fighting cocks used for any purpose [1].
Prevalence	The proportion of a population with a specific characteristic in a given time period.
Salmonella enterica, non-typhoidal (NT *S. enterica*)	In this research report, this refers to serovars in the genus *Salmonella*, species *enterica* and subspecies *enterica* (I) that are associated with foodborne disease. Examples of these serovars are *S.* Enteritidis and *S.* Typhimurium. Non-typhoidal refers to all serovars excluding Typhi, Paratyphi A, Paratyphi B, Paratyphi C, or Sendai [2].
Serovar	A grouping of micro-organisms based on their cell-surface antigens.
Small-scale farm	A farm that produces livestock or agriculture in a small area of land without advanced equipment or technology. Small-scale farms generally have a lower output compared to the commercial sector.
Zoonosis	A disease of animals that may be transmitted to humans.

CHAPTER 1 - INTRODUCTION

This chapter presents an overview of foodborne disease related to *Campylobacter* spp. and non-typhoidal *Salmonella enterica* (NT *S. enterica*) serovars. The chapter focuses on the disease epidemiology in developing countries. The public health impact of foodborne disease is discussed. A critical review of the literature, synthesising studies of the prevalence of *Campylobacter* spp. and NT *S. enterica* serovars in poultry and poultry products worldwide; and associated farm biosecurity and management factors is then presented. This is followed by the aim and objectives of the research study.

The chapter headings are:

1.1 Background

1.1.1 Foodborne campylobacteriosis and salmonellosis

1.1.2 Public health importance of campylobacteriosis and salmonellosis

1.2 Literature Review

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1.2.2 Prevalence studies of *Campylobacter* spp. and non-typhoidal *Salmonella enterica* serovars in poultry and poultry products

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1.3 Problem Statement

1.4 Justification

1.5 Research Question

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1.7 Objectives

1.1 Background

1.1.1 Foodborne campylobacteriosis and salmonellosis

Foodborne diseases including campylobacteriosis and salmonellosis compromise food safety and contribute to morbidity and mortality globally and in Africa [3–7]. The World Health Organisation (WHO) estimated that 582 million cases of foodborne illness occurred worldwide in 2010, resulting in 25.2 million disability adjusted life years (DALYs) lost [3]. Despite a high burden of disease, a relatively low estimate of worldwide mortality due to foodborne disease was reported by the WHO, at 5 deaths per 100 000 population [3]. The public health threat posed by foodborne disease is not uniform in all nations as regional mortality rates vary widely [3]. Africa had a disproportionately high death rate due to foodborne disease (14 deaths per 100 000 population), compared to a low of 0.5 deaths per 100 000 population in Europe [3].

Second only to viral causes, bacterial pathogens predominate in foodborne disease cases worldwide [5,8]. *Campylobacter* spp. and non-typhoidal *Salmonella enterica* (NT *S. enterica*) serovars are among the most commonly isolated zoonotic bacteria associated with foodborne disease [3,5,6]. In 2010, 95.6 million campylobacteriosis cases and 78.4 million non-typhoidal salmonellosis cases occurred globally [3]. These ubiquitous pathogens are associated with substantial morbidity [3,5]. It is estimated that foodborne *Campylobacter* spp. and NT S. *enterica* serovars resulted in a combined 4.3 million DALYs lost globally in 2010 [3]. Foodborne diseases may also cause society to incur considerable economic losses [9,10]. The average cost per foodborne disease case in the United States was estimated at $2 422, while total annual costs specific to *Campylobacter* spp. and non-typhoidal *Salmonella* amounted to $7.9 million and $6.5 million respectively [9].

Aetiology and epidemiology

Campylobacter spp. are gram-negative bacteria in the genus *Campylobacter* and family *Campylobacteraceae*. Several *Campylobacter* spp. may cause disease in humans, animals or both. *Campylobacter jejuni* and *Campylobacter coli* are commonly implicated species in foodborne disease cases [11–13] and *C. jejuni* is the predominant species in most geographic areas [12]. In 2017, 84.4% of *Campylobacter* cases with species information in the European Union (EU) were identified as *C. jejuni* and 9.2% were reported as *C. coli* cases [4]. Although the clinical signs caused by either species are often indistinguishable, *C. coli* may cause up to

25% of all campylobacteriosis infections [12]. Transmission of *Campylobacter* spp. from environmental sources or through horizontal transmission results in colonisation of the caecum, cloaca, large intestine and jejunum [14]. Once colonised, broilers act as a bacterial reservoir, contaminate the rearing environment and transmit *Campylobacter* spp. horizontally throughout the flock. Poultry carcasses may become contaminated with intestinal microflora during the slaughter process [14]. Although *Campylobacter* spp. will not proliferate outside of the digestive tract of warm-blooded animals, these microbes survive for several weeks in food products, especially if stored at low temperatures [11,12]. In contrast to NT *S. enterica* serovars, vertical transmission through eggs is not relevant to the epidemiology of the disease [15].

Salmonella enterica subspecies *enterica* is an intracellular pathogen of warm-blooded mammals and comprises more than 2000 serovars [16]. These gram-negative bacilli of the genus *Salmonella* in the family *Enterobacteriaceae* are important contributors to the worldwide foodborne disease burden [17]. *S. enterica* serovars may be classified as typhoidal or non-typhoidal. Typhoidal serovars are specialised pathogens that are restricted to human hosts and have no animal reservoir. Human infection results in systemic disease known as enteric fever or typhoid, mainly in developing countries [16]. Non-typhoidal serovars occur worldwide and are generalised pathogens with a broader host range of animals and humans [16]. Of the NT *S. enterica* serovars, *S.* Enteritidis (SE) and *S.* Typhimurium are the most frequently reported serotypes associated with foodborne disease [18]. Foodborne disease caused by NT *S. enterica* serovars results from consumption of contaminated animal-derived products, most notably eggs, fresh produce or poultry products. Infection may also occur from direct contact with an infected animal or more rarely from person-to-person transmission [17,19]. *Salmonella* serovars that are specific to poultry (*S.* Gallinarum and *S.* Pullorum) are not included in the scope of this research.

Clinical symptoms

Individuals affected by foodborne campylobacteriosis or non-typhoidal salmonellosis present with gastroenteritis of varying severity [11,25,26]. Campylobacteriosis symptoms include fever, abdominal cramps, vomiting, dehydration and diarrhoea which may be bloody. Usually the disease will resolve after five to seven days but there is risk of severe complications including septicaemia, peripheral neuropathies, arthritis and cardiovascular abnormalities [11,12,27]. Guillain-Barré syndrome is associated with *C. jejuni* infection specifically [12,13].

Foodborne salmonellosis presents clinically as diarrhoea, abdominal cramps and fever. In some cases it may progress to bacterial meningitis, reactive arthritis or endocarditis [11,21]. More severe or fatal cases of campylobacteriosis and salmonellosis may occur in children under five years of age and in immune-compromised or elderly individuals [21,26,27].

Prevalence, distribution and risk factors

Prevalence data for human campylobacteriosis in developing countries is limited, in part by the lack of dedicated surveillance systems [13,28], under-reporting and non-specific disease symptoms [29]. A number of longitudinal studies have been conducted. A Malawian study conducted over a ten-year period detected *Campylobacter* spp. in 21% of children hospitalised with diarrhoea [30]. Similarly, in Kenya, a cohort study isolated *Campylobacter* spp. from 5% of children who died in hospital due to diarrhoea. Notably, NT *S. enterica* was isolated from 20% of the mortalities [31]. Prevalence of *Campylobacter* species in children aged less than five years in Ethiopia was estimated at 10% using meta-analysis techniques [32]. These African studies suggest that *Campylobacter* is commonly detected in children suffering from diarrhoea in multiple African states [30–32].

The global and regional burden of non-typhoidal salmonellosis has been investigated by multiple researchers using various methods [33–36]. A recent systematic review synthesised data from 35 studies and estimated the global incidence of non-typhoidal salmonellosis in 2017 at 7.5 cases per 100 000 person-years. Sub-Saharan Africa had the highest incidence of all regions at 34.5 cases per 100 000 person-years [35]. It is estimated that 93.8 million gastrointestinal illnesses (of which 80.3 million are foodborne) caused by NT *S. enterica* serovars occur annually [34]. African incidence estimates ranged from 1.4 to 2520 cases per 100 000 population per year, with higher incidence rates occurring in children and in inhabitants of rural areas [36].

Risk factors for campylobacteriosis and salmonellosis may vary in high versus low-to-middle income countries [37–42]. In high-income countries, important risk factors for salmonellosis include eating raw or undercooked poultry meat and eggs [41]. In contrast, in developing countries, using pit latrines or rivers and canals as toilets and drinking water from a non-piped source are associated with disease [39]. Case-control studies in the Netherlands and Tanzania demonstrated that foodborne transmission involving consumption of poultry and undercooked meat were the greatest risk factors for campylobacteriosis [38,40,42]. In a systematic review and meta-analysis carried out using eight observational studies conducted in Ethiopia,

consumption of animal products, illiterate mothers and the status of mothers' personal hygiene were significantly associated with the prevalence of *Campylobacter* spp. in children aged under five [32]. Contact with farm animals [38] and domestic animals [32] may also increase the risk of campylobacteriosis. Risk factors for foodborne disease are diverse and highlight that animals and animal products are important sources of disease, regardless of socioeconomic conditions.

Bacterial colonisation of poultry

Campylobacter spp. are commensal organisms in poultry. Therefore, birds that are colonised with the bacteria are clinically healthy and are asymptomatic [15,20]. Isolated studies have suggested that young poultry birds with intestinal bacterial colonisation may experience diarrhoea and weight loss with mild gastrointestinal pathological lesions [15]. Similarly, poultry that are colonised with generalist NT *S. enterica* serovars (this excludes the poultry-specific agents *S.* Gallinarum and *S.* Pullorum) are sub-clinically infected since these microbes are commensals in poultry [21]. There is no evidence that production at the farm-level is reduced by colonisation of poultry with generalist NT *S. enterica* serovars or *Campylobacter* spp. The poultry farm presents a critical control point for the reduction of human foodborne campylobacteriosis and salmonellosis. Poultry farmers may be able to reduce the incidence of human disease by limiting colonisation of poultry at the farm stage of the poultry production chain [22].

Disease surveillance in small-scale broilers

Many small-scale broiler farmers do not produce for export purposes, for which regular *Salmonella* monitoring is a requirement [23]. These producers may also bypass the use of registered abattoirs where meat inspection is conducted [24]. Therefore, there is limited justification to maintain broilers free of NT *S. enterica* and *Campylobacter* spp. in small-scale production systems that cater to informal local markets. It is challenging to incentivize broiler farmers to control NT *S. enterica* serovars and *Campylobacter* spp. as bacterial colonisation does not directly reduce production [15] or profit. To promote food safety in small-scale production systems, the production of broilers free of NT *S. enterica* serovars and *Campylobacter* spp. should enable improved access to high-quality markets and an associated increased profitability.

1.1.2 Public health importance of campylobacteriosis and salmonellosis

Campylobacter spp. and NT *S. enterica* serovars are established causes of zoonotic foodborne disease and pose a risk to human health worldwide [3,4,11,25]. The WHO's 2010 data synthesis on the global and regional disease burden of foodborne diseases established that the rate of DALYs for foodborne diseases was highest in the African region [3]. Of the 22 foodborne diseases considered, those caused by invasive and non-invasive NT *S. enterica* serovars resulted in the greatest (4.07 million) foodborne DALYs in 2010. *Campylobacte*r pathogens caused 2.1 million foodborne DALYs in 2010 and 1390 illnesses per 100 000 person years globally [3]. The impact of these pathogens was reiterated in a similar study focused on the high-income region of the United States. Both NT *S. enterica* serovars and *Campylobacter* spp. resulted in considerable DALYs lost annually (32900 and 22500 DALYs respectively) [5]. Moreover, vulnerable populations such as children aged five and under are more likely to experience severe disease caused by foodborne pathogens and are disproportionately affected by foodborne disease, with 38% of an estimated 582 million foodborne illnesses occurring in children under five years [3]. Economic fallout resulting from campylobacteriosis and salmonellosis may be substantial. It is estimated that the food-pathogen pairs that incurred the highest costs in the United States were *Campylobacter* spp. in chicken ($6.9 billion) and *Salmonella* serovars in poultry ($2.8 billion) [9]. Morbidity and mortality due to campylobacteriosis and salmonellosis is associated with social and economic losses and may burden the healthcare sector [3,11].

Further negative implications of foodborne disease relate to antimicrobial drug resistance (AMR) [43,44]. The routine use of fluoroquinolones and other antibiotics in poultry farms has been associated with increased AMR of *Campylobacter* spp. and NT *S. enterica* serovars in both humans and animals [13,21,45–47]. *Campylobacter* spp. are documented to continuously develop new mechanisms for AMR [48,49]. Some of these mechanisms lead to resistance to single drugs but multi-drug resistance has also developed and cases of drug-resistant human campylobacteriosis are an emerging food safety risk [48,50]. Effective treatment of salmonellosis in humans is limited by resistance of the pathogen to certain antimicrobials [51,52]. In a study in the United States, 2.8% of NT *S. enterica* isolates were resistant to clinically important fluoroquinolones and 2.5% of isolates were resistant to a third-generation cephalosporin [51]. Overall, in the United States and Europe there is a low to moderate level

of antimicrobial resistance in NT *S. enterica* isolates however certain serovars have demonstrated an increasing trend of single or multi-drug resistance [51,52].

1.2 Literature Review

1.2.1 Literature search strategy

PubMed and Google Scholar databases were searched on a regular basis between July 2020 and October 2021. Material discussing foodborne disease caused by *Campylobacter* species and NT *S. enterica* serovars, prevalence studies of these bacteria in poultry or animal-derived food products were retained. Publications that studied risk factors or associations with these pathogens in food-producing animals or on farms were also extracted. Material was restricted to English publications. Search terms that were used included: ('*Campylobacter*' OR 'Campylobacteriosis' OR '*Campylobacter jejuni*' OR '*Campylobacter coli*') OR ('*Salmonella*' OR 'Salmonellosis' OR '*S. enterica*' OR '*Salmonella* Enteritidis*'* OR *'*non-typhoidal *Salmonella*) AND ('prevalence' OR 'burden' OR 'presence' OR 'risk factor' OR 'factor' OR 'correlates' OR 'incidence' OR 'association') AND/OR ('poultry' OR 'broiler' OR 'chickens' OR 'poultry products' OR 'chicken meat' OR 'food-producing' OR 'farm'). Different permutations of these terms were used. The abstracts were used to select literature that would be relevant to the current study. The full text papers were downloaded and managed using Mendeley reference software. Studies that were included in the literature review were in most cases observational studies, meta-analyses or systematic reviews about the prevalence of *Campylobacter* spp. or NT *S. enterica* serovars in poultry or poultry products and factors associated with the presence or absence of disease.

1.2.2 Prevalence studies of *Campylobacter* spp. and non-typhoidal *Salmonella enterica* serovars in poultry and poultry products

A number of studies estimating the prevalence of both *Campylobacter* spp. and NT *S. enterica* serovars in food producing animals and animal products have been conducted in countries across all income levels [4,47,53–72]. The studies were predominantly conducted in the large-scale, commercial poultry sector with knowledge gaps concerning the small-scale poultry sector [56–60,71,72]. The prevalence studies were conducted between 2001 and 2020 with small (n=7) and larger (n=2835) sample sizes [61,62]. Poultry at different stages of the production chain including at the farm-level, abattoir, processing plant and in the retail, environment were sampled. Since prevalence estimates have been found to vary at these

different stages [73,74], it is difficult to compare the prevalence findings for *Campylobacter* and NT *S. enterica* serovars. Additionally, these prevalence studies utilised a variety of sample types including cloacal and caecal contents, carcass swabs and washes, duodenal samples, faeces or litter and environmental samples. Isolation of *Campylobacter* spp. was not equally successful for all types of samples. For example, *Campylobacter* spp. was detected from a greater proportion of broilers using laboratory testing of cloacal swabs in charcoal Amies medium compared to testing of caecal contents collected during necropsy [75]. Importantly, multiple studies have demonstrated that PCR methods to screen and confirm *Campylobacter* spp. from a variety of samples are superior to culture techniques [76–78]. Therefore, prevalence studies that relied only on traditional culture methods may present under-estimates. Due to the varying sample types and diagnostic tests used, it has been challenging to estimate a global prevalence statistic of either pathogen in broiler populations.

Broadly, *Campylobacter* spp. are highly prevalent in broiler populations in developing and developed nations (see Table 1.1, tabulated in order of descending prevalence). A combination of bacterial culture and polymerase chain reaction testing of gastrointestinal samples including caecal contents and cloacal swabs detected a prevalence range of 5.9%-100% [55,56,58–60,64,65,71,76,79]. Prevalence findings from faecal samples had a more narrow range of 7%-78% [57,61,62,64,72,80]. Despite these findings, it is not known if small-scale production systems have comparable *Campylobacter* spp. burdens.

Table 1.1: *Campylobacter* spp. prevalence estimates in poultry and poultry products from observational studies

Country	Year	Sample	Sample size	Prevalence	Reference
Brazil	2010	Farm litter	40	100%, (40/40)	[71]
		Pooled caecal content	40	100%, (40/40)	
		Carcasses	40 with faecal contamination	17.6%, (7/40) *(C. jejuni)*	
			40 without faecal contamination	58.8%, (23.5/40) *(C. jejuni)*	
France	2008	Pooled caeca and carcasses	425 carcasses	87.5%, (372/425)	[68]
			425 pooled caecal samples	77.2%, (328/425)	
South Africa	2016	Faecal samples	100	78%, (78/100)	[80]
Iran	2009	Caecal swabs	100	76%, (76/100)	[60]
Belgium/ Denmark	2002-2004	Gastrointestinal tract	56 broiler flocks	73%, (41/56)	[69]
Morocco	2017	Cloacal swabs	105	71.4%, (75/105)	[79]
South Africa	2008-2009	Caecal contents	56 rural chickens	68%, (38/56)	[55]
			140 commercial free-range chickens	47.1%, (66/140)	

Country	Year	Sample	Sample size	Prevalence	Reference
			133 industrial broilers	47.4%, (63/133)	
Lebanon	2016	Caecal contents	227	67%, (152/227)	[65]
		Neck skin	227	17.2%, (39/227)	
Kenya (Kibera)	2018	Chicken meat (retail)	28	64%, (18/28)	[61]
		Faeces/litter	7	43%, (3/7)	
Kenya (Dagoretti)	2018	Chicken meat (retail)	25	60%, (15/25)	[61]
		Faeces/litter	18	33%, (6/18)	
Thailand	2012-2014	Caecal samples	442	57%, (252/442)	[56]
New Zealand	2007	Whole carcasses (skin)	163 carcasses	44.8%, (73/163)	[70]
Bangladesh	2019	Cloacal swabs	84 pooled cloacal swabs from 84 broiler farms	40.5%, (34/84)	[59]
Spain	2010-2012	Cloacal swabs	2221	38.1%, (846/2221)	[58]
South Africa	2003	Fresh and frozen chicken carcasses	99 carcasses	32.3%, (32/99)	[54]
United States of America	2010-2011	Faecal samples	400	29.5%, (118/200)	[72]
Australia	2016	Faecal and environmental samples	1856	28.3%, (526/1856)	[57]
Denmark	2009-2010	Faecal samples	2835 flocks	14%, (388/2835)	[62]
Iceland	2016-2018	Faecal and caecal samples	857 flocks	7%, (41/857)	[64]
South Africa	Unknown	Cloacal swabs	408	5.9%, (24/408)	[76]

Campylobacter spp. have been isolated from numerous food-producing animals and animal-derived food products [63,67]. A recent meta-analysis of observational studies conducted between 1980 and 2019 in predominantly high-income countries in Europe and North America analysed the global pooled prevalence of *Campylobacter* spp. in food products of animal origin [67]. Poultry meat was found to be the food source primarily responsible for human exposure to the pathogen. The highest prevalence of *Campylobacter* spp. in sampled livestock (including cattle, pigs, broilers, hens, goats and sheep) was detected in broilers. Broiler carcasses and livers had a prevalence of 52.3% and 65.5% respectively. The meta-analysis suggested that the highest prevalence (14.1%) of *C. coli* was identified in broilers and that prevalence of *C. jejuni* in broiler meat was 33.7% (95% CI 30.7%-36.8%) [67]. Importantly, studies that were included utilised varying diagnostic methods (morphological identification, biochemical or PCR testing) which may differ in sensitivity and bias the results.

Observational studies that estimated the prevalence of NT S. enterica serovars in broiler poultry have generally isolated NT S. enterica at a lower level than Campylobacter spp. (see Table 1.1 and Table 1.2, tabulated in order of descending prevalence). Developing countries had a high burden of NT S. enterica-colonised or contaminated broilers, (38.6% prevalence in Senegal [81] and 42% prevalence in Bangladesh [82]) but, there is evidence that some high-income countries have comparably high prevalence rates (22% prevalence in Canada [83] and 13% prevalence in a combined survey of Belgium and Denmark [69]). NT S. enterica prevalence estimates ranged between 0%-42% and were determined from a range of samples (including cloacal swabs, carcasses and litter) (see Table 1.2) using diverse diagnostic methods [82,84]. Gaps regarding NT *S. enterica* prevalence at the on-farm rearing stage and specifically in small-scale production systems remain.

Table 1.2: NT *S. enterica* prevalence estimates in poultry and poultry products from observational studies

Country	Year	Sample	Sample size	Prevalence	Reference
Bangladesh	2016	Cloacal swabs	60	42%, (25/60)	[82]
Senegal	Unknown	Pooled faecal samples	57	35.1%, (20/57)	[81]
		Chicken carcass skin	57	38.6%, 22/56	
		Chicken carcass muscle	57	29.8%, 17/56	
		Chicken meat (restaurant)	42	14.3%, (6/56)	
Canada	2012-2013	Chicken carcasses and breast/thigh pieces	2732	22%, (597/2732)	[83]
South Africa	Unknown	Fresh and frozen chicken carcasses	99	19.2 %, (19/99)	[54]
Nigeria	2019	Faecal and environmental samples	558	15.9%, (89/558)	[85]
Trinidad	Unknown	Carcass swabs and carcass rinse fluids	450	14.2%, (64/450)	[86]
Belgium/ Denmark	2002-2004	Gastrointestinal tract	56 broiler flocks	13%, (7/56)	[69]
Iran	2018	Faecal samples	110	11,8%, (13/110)	[87]
Zimbabwe	2003-2005	Pooled cloacal swabs	2833	10%, (283/2833)	[88]
Nepal	2019	Faecal and environmental samples	288	9%, (26/288)	[89]
United States of America	2010-2011	Faecal samples	400	8.8%, (35/400)	[72]
France	2008	Pooled caeca and carcasses	425 batches	7.5%, (32/425)	[68]
Brazil	2010	Farm litter	40	5%, (2/40)	[71]
		Pooled caecal content	40	0%, (0/40)	
		Carcasses	120	0%, (0/120)	
Ethiopia	2013-2014	Pooled fresh faecal droppings	549	4.7%, (26/549)	[90]
South Korea	2013	Raw poultry meat	80	3.7%, (3/80)	[91]
Ethiopia	2017-2018	Cloacal swabs, faecal droppings, feed samples and floor swabs	836	2.9%, (24/836)	[84]
New Zealand	2007	Whole carcasses	163	0%, (0/163)	[70]

European prevalence estimates for *Campylobacter* spp. and NT *S. enterica* serovars in animals and animal products have been reported by multiple researchers [4,68,69,92]. The European Food Safety Authority (EFSA) reviewed data from European Union-coordinated zoonoses monitoring systems to determine a 37.4% *Campylobacter* prevalence in fresh broiler meat and 12.3% prevalence in live broilers in 2017. At flock level, the European Union had a 3.31% *Salmonella* prevalence [4]. The EFSA findings present an overall picture of the burden of disease on the continent. Cross-sectional studies limited to a single European state offer higher prevalence estimates for both pathogens. French and Danish research produced comparable prevalence results for *Campylobacter* spp. of 77.2%-87.5% prevalence depending on the type of sample in France [68] and 73% flock-level prevalence in Denmark [69]. NT *S. enterica* prevalence findings were 7.5% and 13% in France and Denmark respectively [68,69]. The Danish study employed multi-sampling techniques (duodenum, crop and caecum) in contrast to other prevalence studies [69]. The researchers emphasised that this multi-sampling technique was necessary to detect NT *S. enterica* and may be related to the slightly higher NT *S. enterica* prevalence estimate compared to others in Europe [4,69]. Sampling strategies such as using slaughter batches as the sampling unit and pooling samples for analysis [68] may have led to an over-estimation of the true bacterial prevalence. The available research indicates that *Campylobacter* spp. are present at a greater prevalence than NT *S. enterica* serovars in European broiler populations.

The selection of sample type or the stage of broiler production at which sampling occurs may affect prevalence findings in observational studies. Higher *Campylobacter* spp. prevalence estimates were found based on whole carcass sampling (87.5%) versus caecal sampling (prevalence of 77.2%) [68]. This may illustrate that contamination of the skin of the carcass occurs in the slaughterhouse. Evisceration of carcasses has been shown to contaminate neighbouring carcasses and can contaminate the following slaughter batches which may have entered the slaughterhouse disease-free [46,68]. The slaughter process itself, rather than true colonisation of the digestive tract of poultry may cause an apparent increased prevalence of *Campylobacter* in samples tested at the point of slaughter. Conflicting results concerning the effect of faecal contamination on prevalence estimates were reported from Brazil [71]. Caecal contents had a prevalence of 100% and carcasses without obvious faecal contamination had a higher *Campylobacter jejuni* prevalence (58.8%) than those with obvious faecal contamination (17.6%). In contrast, prevalence estimates for *Campylobacter coli* were lower and suggested that faecal contamination increased the bacterial burden: 70.6% based on caecal samples,

11.6% in carcasses with macroscopic faecal contamination and 9.8% in carcasses without visible faecal matter [71]. These findings demonstrate the importance of the slaughter process in determining the risk of exposure to the consumer. These existing data also point to the gaps in knowledge of pre-slaughter bacterial prevalence and on-farm bacterial loads.

Investigation of the prevalence of *Campylobacter* spp. and NT *S. enterica* serovars in chicken products in the retail or restaurant chain has established that chicken products pose a risk to food safety. Heavy burdens of *Campylobacter* spp. were detected in Kenyan (prevalence of 60-64%) [61], Thai (prevalence of 56.8%) [93] and Australian (prevalence of 84%) [94] chicken meat and offal products. A high proportion (70.7% and 44.8% respectively) of raw chicken samples in the United States [95] and New Zealand [70] were also contaminated with *Campylobacter* spp. These prevalence findings do not necessarily measure colonisation with *Campylobacter* spp. Rather, the results are probably an indication of contamination of the carcass which may occur at slaughter, during processing or in a retail setting. Similar to prevalence studies conducted at the abattoir or processing plant, NT *S. enterica* serovars were detected at a lower prevalence than *Campylobacter* spp. in retail or restaurant chicken products. Chicken served at street restaurants in Senegal had a NT *S. enterica* prevalence of 14.3% [81] and NT *S. enterica* was not detected in 163 retail chickens in a New Zealand based study [70]. Research focused on the retail stage of production fails to account for possible on-farm sources of bacteria.

The bacterial burden of broiler poultry may fluctuate depending on the stage of production. A recent systematic review and random-effects meta-analysis investigated the prevalence of *Campylobacter* and NT *S. enterica* in the farm environment, processing and retail stages in the United States [74]. *Campylobacter* prevalence was estimated at 59.2% at the retail level. The highest prevalence of *Campylobacter* was found during processing (60.9%-97.9%) and environmental samples at farm level had the lowest prevalence at 15.8%. NT *S. enterica* prevalence in these three stages (farm environment, processing and retail) followed a different pattern with the farm environment at a higher prevalence (22.9%) than the retail stage (19%). Processing-level prevalence estimates ranged from 14.3%-68.6% [74]. The meta-analysis was limited since some study groups included a low number (n=2) of studies in the analysis. Although there was high heterogeneity between the study groups, the meta-analysis model was fitted with multiple moderating variables to account for this concern.

1.2.3 Studies of *Campylobacter* spp. and non-typhoidal *Salmonella enterica* prevalence in poultry and poultry products limited to the African continent

Animal reservoirs of *Campylobacter* spp. or NT *S. enterica* serovars are recognized to be highly prevalent in the African region. A random effects meta-analysis published in 2019 addressed the prevalence of both *Campylobacter* spp. and NT *S. enterica* serovars in food-producing animals and food products in 27 African countries [66]. The research indicates that *Campylobacter* could be isolated from 37.7% (95% CI 31.6–44.3) of all poultry samples. Isolation of *Campylobacter* was highest (73.8%) from external poultry samples (defined as skin or feathers) and lowest based on poultry meat or organs (21.3%). Gastrointestinal samples yielded a prevalence of 40.2%. Regionally, Central Africa had the highest prevalence (91.2%) while Northern Africa had a prevalence of only 24.2%. Contamination of the external carcass by *Campylobacter* spp. from other animals or faecal matter during slaughter or possibly pre-slaughter can be reinforced by the meta-analysis' findings of higher prevalence from external samples as opposed to all other sampling areas. NT *S. enterica* serovars were detected in 13.9% (95% CI 11.7–16.4) of poultry samples. Similarly, external poultry samples had the highest prevalence of NT *S. enterica* (28.5%) and poultry meat or organs had the lowest prevalence (13.2%). The prevalence in gastrointestinal samples was similar at 13.4%. NT *S. enterica* prevalence was higher in Southern Africa than in Northern Africa with prevalence values of 28.2% and 10.5% respectively [66]. The meta-analysis included 247 studies, 25 of which were carried out before 2000 and four of these were published nearly 70 years ago. However, the remaining 222 studies took place between 2000 and 2016. The studies that were analysed employed various laboratory techniques and some diagnostic procedures were not fully explained. Therefore, comparability of study findings is limited. While *Campylobacter* spp. and NT *S. enterica* serovars were detected throughout the continent, this study emphasises that these pathogens are not equally prevalent in all African countries.

A comprehensive review of studies estimating the prevalence of *Campylobacter* spp. in food-producing animals and in humans by African region and country also illustrated the variability of *Campylobacter* prevalence by geographic location [63]. This review did not include NT *S. enterica* prevalence estimates nor did the authors undertake meta-analysis. The studies reviewed collected various types of poultry samples including fresh and frozen chicken from the retail environment and carcasses at point of slaughter [63]. African estimates of *Campylobacter* prevalence in poultry products range from 9.6% (n=680) in Egypt to 90%

(n=150) in Cameroon [63]. Though all of the studies included in the review used culture techniques for isolation of *Campylobacter* spp., prevalence estimates varied widely [63].

South African studies that focus explicitly on *Campylobacter* spp. or NT *S. enterica* bacterial isolation from poultry or poultry products are limited. A local study to examine the incidence of NT *S. enterica* serovars in food-producing animals, animal feed and environmental sources isolated NT *S. enterica* serovars in 5% (9031/180298) of samples [53]. SE was the most commonly isolated serovar – with 21.5% of the isolated NT *S. enterica* serovars detected from poultry on farms, poultry meat and poultry houses [53]. In an observational study limited to poultry carcasses in Gauteng Province, *Campylobacter* was isolated from carcass rinse fluids in 32.3% of sampled fresh and frozen retail chickens and NT *S. enterica* was detected in 19.2% of samples. Importantly, this prevalence study may have been limited by the small sample size (99 samples) included in the analysis [54]. This study was conducted at the retail level and did not address the prevalence of these pathogens at farm-level or at the abattoir specifically.

Data concerning bacterial prevalence of *Campylobacter* spp. in South Africa's commercial poultry sector is sparse but more available than that of the small-scale poultry sector. A cross-sectional study conducted in KwaZulu-Natal Province, South Africa compared the prevalence of *Campylobacter* spp. among chickens in different production systems (rural production, commercial free-range systems and industrial broiler production) [55]. The findings indicated that chickens reared in a rural setup had the highest prevalence of *Campylobacter* (68%) while commercial free-range chickens and industrial broilers had lower and equal prevalence estimates of 47% [55]. Though this study was limited by a small sample size (56 rurally-produced chickens, 140 samples from the commercial free range farm and 133 samples from industrial broilers) it offers a unique perspective on the effect of differing production systems and related farm management practices on the prevalence of the pathogen in broilers [55]. No similar study that contrasts production systems could be identified for comparison. Discordant prevalence estimates of *Campylobacter* spp. in commercial chickens have been estimated in KwaZulu-Natal Province (78% based on 100 faecal swabs) [96] and North West Province (5.9% from 408 cloacal swabs) [76]. The available research indicates that South African poultry and poultry products are a reservoir of *Campylobacter* spp. and NT *S. enterica* serovars and act as a human disease exposure risk [53–55,76,96]. Prevalence data specific to the small-scale poultry industry in South Africa is lacking.

1.2.4 Biosecurity and management practices associated with colonisation of poultry by *Campylobacter* spp.

In principle, the risk of bacterial contamination of poultry carcasses during later stages of processing may be reduced by implementing strategies at the primary stages of production which take place at the farm level [97]. Specifically, faecal contamination of carcasses at the abattoir may be prevented if the presence of *Campylobacter* bacteria in poultry faeces is addressed at the source [98]. It is estimated that the prevention of high rates of *Campylobacter* spp. colonization in poultry has the potential to reduce human cases related to poultry meat consumption by 7-10% [99].

Numerous epidemiological studies have examined the effect of biosecurity on colonisation of poultry with *Campylobacter* spp. [45,50,58–62,97,99–102]. However, the majority of these studies were conducted in high-income countries in Europe and North America. The literature review could not identify a South African study. The literature that examines the relationship between biosecurity practices and *Campylobacter* prevalence is largely focused on the commercial poultry sector [58,60,62,99–101] as opposed to small-scale or backyard operations. A single study of small-scale chicken farms in a low to middle income peri-urban area was identified [61].

Various biosecurity and management practices associated with an increased risk of colonisation with *Campylobacter* spp. are presented in the literature. These include: increasing age of birds in the flock [45,58,59,102], age at slaughter greater than 45 days [60] and 35 days [62], increasing number of poultry houses on the farm [45,50,100,102], increasing age of the poultry house building [59,62,101], the practice of partial depopulation [45,58,102], increased number of farm workers in the poultry houses [62,102] and increased number of personnel visits to the houses per day [102]. Poor cleaning and disinfection of poultry houses and the surrounds [50,59,102] as well as a non-cement floor material that is not easy to clean [61] were also associated with an increased risk of *Campylobacter* colonisation. Less than 10 years of farming experience and the use of rice husk as a litter material were significantly associated with *Campylobacter* prevalence in a 2019 Bangladeshi cross-sectional study, although causality cannot be determined using this study design [59]. It was argued that the presence of other livestock on the farm or nearby [45,58,100] and rodents or insects in the poultry house [45,58] are factors associated with an increased risk of bacterial colonisation.

A number of exposures have had contradictory effects on *Campylobacter* spp. colonisation of poultry in reported literature. Examples include the presence of other livestock on the farm [50,102], the presence of wildlife [50,102], pets (dogs and cats) [50,58,102], rodents and rodent control, insects [50,102] and length of time the poultry houses are left empty between flocks (downtime) [50,100–102]. Additional management practices with inconclusive predictive or protective effects include: water source and water disinfection [45,50,58,100,102], flock size and stocking density [45,50,59,102]. The heterogeneity of these findings may be related to unmeasured confounders (possibly broiler breed and breed-related disease susceptibility, or effect of in-feed antibiotics), varying laboratory diagnostic methods and different study environments (namely commercial farming versus informal production). Aspects of methodological design such as sample type and stage of production would also possibly contribute to these conflicting findings.

The literature highlighted that scientific knowledge of biosecurity interventions that could contain the spread of *Campylobacter* was inconsistent. Practices that could reduce the risk of *Campylobacter* colonisation are mostly related to the use of a hygiene barrier or entrance room. This is a station at which hand-washing, house-specific boots and clothes, overshoes and foot baths are used [45,50,58,101,102]. Conversely, foot baths may also increase the risk of colonisation if not properly used, replenished and maintained [45]. The all-in-all-out system in place of partial depopulation [45,50,102], use of dedicated equipment for each poultry house [50], staff training and limited access to key personnel only [50] are also important biosecurity measures to reduce colonisation in broilers [50]. A review of biosecurity-related literature suggested that vehicle disinfection and gates for each poultry house may be important in reducing the prevalence of *Campylobacter* on the farm [50]. An Iranian cross-sectional study conducted on commercial broiler farms suggested that the use of antibiotic drugs in the first two weeks of production and an increasing level of education of the farmer were protective factors [60]. Though many possible sources of disease on the farm were identified, evidence for control was conflicting. No clear target for improved disease control at the farm-level was identified [45,50,102].

1.2.5 Biosecurity and management practices associated with colonisation of poultry by non-typhoidal *Salmonella enterica* serovars

Given that the epidemiology of NT *S. enterica* serovars and *Campylobacter* spp. in broiler production systems differ, biosecurity and management practices of importance to these diseases may also diverge. Limited research is available concerning NT *S. enterica* serovars

and associated biosecurity and management practices. The available literature primarily discusses commercial poultry farming which generally operates with greater resources than the small-scale poultry industry. Most studies discuss vaccination as a tool for *Salmonella* prevention without addressing the role of basic biosecurity in the spread of *Salmonella* serovars [103–105]. Totton et al.'s [103] random effects meta-analysis of the effect of biosecurity and vaccination on NT *S. enterica* serovars in broiler poultry was limited by the minimal number of relevant studies available for analysis. Of the 22 studies addressing vaccination against NT *S. enterica* serovars, the majority were conducted in high income countries in Europe and North America. Vaccination with a modified-live *S.* Typhimurium vaccine (investigated in nine studies) was found to reduce the risk of colonisation in broilers [104]. A single observational study in Canada that investigated risk factors for colonisation with NT *S. enterica* serovars in broilers, found that the odds of bacterial colonisation for chicken houses that were not permanently locked was 2.6 times the odds of colonisation for chicken houses kept permanently locked [106]. A similar study in the Netherlands identified that NT *S. enterica* prevalence increased with increasing flock size [73]. The feed supplier also had a significant effect on prevalence, suggesting that contaminated feed material is an important risk factor [73]. Additional factors associated with colonisation and contamination with NT *S. enterica* serovars include: a downtime less than one month, having less than five fans in the poultry house, farms with less than four houses [87] and on-farm disposal of waste [85]. In common with findings from studies dealing with *Campylobacter* colonisation [45,58,100], the presence of other livestock on the farm increased the risk of NT *S. enterica* in poultry [85]. Epidemiological research concerning biosecurity and management practices associated with NT *S. enterica* colonisation in broilers is not as comprehensive as that related to *Campylobacter* colonisation. Based on the available literature, farm-level biosecurity and management practices offer important control points which may be used to limit the entry of NT *S. enterica* serovars into the human food-chain. However, specific biosecurity practices relevant to small-scale producers have not been conclusively identified.

1.3 Problem Statement

Colonisation of broiler poultry with *Campylobacter* spp. and NT *S. enterica* serovars compromises food safety and facilitates the transmission of foodborne zoonotic disease to consumers of poultry products [11,21,25–27,46,107]. A limited number of studies have estimated the prevalence of *Campylobacter* spp. or NT *S. enterica* serovars in the South African

commercial poultry population [53–55] and even less research has been conducted in the small-scale broiler sector [55]. Although it is accepted that effective biosecurity practices can reduce harmful bacteria at the farm-level, few data concerning management and biosecurity correlates of disease exist for African-based small-scale poultry production systems.

1.4 Justification

Currently, the prevalence of *Campylobacter* spp. and NT *S. enterica* serovars in small-scale broiler units in South Africa is not known. The local small-scale broiler sector probably presents substantial food safety and public health challenges related to *Campylobacter* spp. and NT *S. enterica* serovars. Baseline prevalence estimates are important for monitoring disease trends and evaluating the effect of disease control interventions in poultry populations. In addition, information on the prevalence of these organisms may be used to guide policies and control measures within this production sector. This would assist to improve the health of small-scale poultry populations and possibly expand poultry product exports. Numerous studies [45,50,97,99,102] in high-income countries and commercial poultry enterprises examined the benefits of biosecurity practices on these diseases in poultry. However, small-scale farms in developing countries have not been the subject of such investigations. The findings of this study could provide evidence for effective and practical biosecurity measures to target important foodborne bacterial pathogens in small-scale broiler production systems. Practical methods to reduce colonisation of birds with pathogens at the farm may be useful in mitigating the risk of foodborne disease in humans [99,107].

1.5 Research Question

Which biosecurity or farm management practices are associated with the prevalence of *Campylobacter* spp. and NT *S. enterica* serovars in small-scale broiler production systems in Gauteng Province, South Africa?

1.6 Aim

This study aimed to estimate the prevalence of *Campylobacter* spp. and NT *S. enterica* serovars in small-scale broiler production systems in Gauteng Province and determine the correlation of

farm biosecurity and management practices with isolation of *Campylobacter* spp. and NT *S. enterica* serovars.

1.7 Objectives

1. Estimate the prevalence of *Campylobacter* spp. and NT *S. enterica* serovars in the small-scale broiler sector in Gauteng Province.
2. Describe the farm-level biosecurity and management practices implemented on small-scale broiler farms in Gauteng Province.
3. Examine the association of implemented biosecurity and management practices with isolation of *Campylobacter* spp. and NT *S. enterica* serovars in small-scale broilers in Gauteng Province.

CHAPTER 2 - METHODS

This chapter provides information about the primary data source and data collection processes. For the present study, the methods used for data management and descriptive analysis of the small-scale farms and biosecurity practices are then presented. Finally, the methods applied for inferential analysis to investigate farm management and biosecurity correlates of *Campylobacter* spp. positivity using logistic regression are indicated.

The chapter headings are:

2.1 The primary data
- 2.1.1 Data source
- 2.1.2 Setting
- 2.1.3 Study population
- 2.1.4 Study sample
- 2.1.5 Sampling strategy
- 2.1.6 Data collection process

2.2 Present study
- 2.2.1 Study design
- 2.2.2 Sampling strategy
- 2.2.3 Precision and power calculation

2.3 Data management

2.4 Exposure variables

2.5 Outcome variable

2.6 Descriptive analysis
- 2.6.1 Study participants
- 2.6.2 Prevalence estimates

2.7 Inferential analysis
- 2.7.1 Univariable analysis
- 2.7.2 Multivariable analysis

2.8 Ethical considerations

2.1 The primary data

The present study is a secondary analysis of the primary data described below.

2.1.1 Data source

The data source was operational data collected by Gauteng Veterinary Services (GVS), Gauteng Department of Agriculture and Rural Development (GDARD). The primary data were not collected for the purposes of a primary scientific study. The data were collected as part of routine GVS disease surveillance activities for *Campylobacter* spp. and *Salmonella* serovars. Routine biosecurity audits were conducted simultaneously at the sampled farms by GVS officials to assess and improve the farming practices on emerging small-scale broiler farms.

2.1.2 Setting

Data were collected from November 2020 to May 2021 across three areas of Gauteng Province (Pretoria, Randfontein and Germiston).

2.1.3 Study population

The study population was all small-scale broiler farms in Gauteng Province that had a flock size of between 100 and 120 000 broilers.

2.1.4 Study sample

GDARD's study sample was 30 consenting small-scale broiler farmers (10 farms each in Pretoria, Randfontein and Germiston). At each farm, 30 broiler birds were sampled, therefore, there were 900 poultry samples in total.

2.1.5 Sampling strategy

No complete register of small-scale broiler farms in Gauteng Province exists. Therefore, the size of the small-scale broiler industry is not known and can only be estimated. A representative sample of small-scale broiler farms were selected from a list of 45 farms known to GDARD using stratified random sampling. Small-scale broiler farms were divided into three strata based on area and 10 farms were randomly selected per area using simple random sampling. At each farm, 30 broiler birds were sampled using simple random sampling.

2.1.6 Data collection process

After the farmer provided written informed consent [Appendix E], a trained GVS veterinary official conducted an inspection of the farm and completed a questionnaire with the farmer using the EpiCollect5 mobile application [Appendix F]. The questionnaire collected demographic information about the farmer and data about farm management and biosecurity practices. A unique reference number was generated for each farm and used to label the laboratory submission form. The veterinary official then collected cloacal samples from 30 broiler birds using swabs with Amies charcoal-based transport medium. The cloacal swabs were kept at a cool temperature and transported to the Agricultural Research Council – Onderstepoort Veterinary Research (ARC-OVR) laboratory on the same day as sample collection. Standard methods for bacterial culture of *Salmonella* (ISO 6579 of 2002) and *Campylobacter* spp. (ISO 10272-1 of 2006) were applied. ISO 6579 of 2002 is used to detect any *Salmonella* serovar and is not limited to NT *S. enterica* serovars. Speciation of positive *Campylobacter* culture results was performed using a real-time polymerase chain reaction (PCR) test. Since these bacteria are not normally present, growth of any *Salmonella* serovar or *Campylobacter* spp. by bacterial culture qualified as a positive result, and no cut-off value was required for interpretation.

2.2 Present study

2.2.1 Study design

A cross-sectional study design was applied for this secondary data analysis.

2.2.2 Sampling strategy

Data collected from all 30 farms (900 birds) were included in the analysis.

2.2.3 Precision and power calculation

The sample size was not calculated since it was predetermined by the primary data. A precision calculation was manually performed using the below formula where p was the expected prevalence and n was the sample size. For this calculation, a prevalence of 75% was assumed. A precision estimate, or measure of uncertainty, of 2.8% was obtained. This suggests that the true prevalence may lie between 2.8% lower or higher than the calculated value.

$$Precision = \pm 1.96 \sqrt{\frac{p\,(1-p)}{n}} \times 100 = \pm 1.96 \sqrt{\frac{(0.25)(0.75)}{900}} \times 100 = 2.8\%$$

Power of the prevalence analysis was calculated with Stata version 15 (StataCorp, College Station, TX, USA) using the power calculator [108]. A moderate design effect of 1.5 was used to account for clustering of birds within farms, therefore, the effective sample size was 300. At a significance level of 5%, the sample had over 90% power (93%) to detect as significant a difference in prevalence of 10% versus 25% between two equally sized groups. When a more conservative design effect of two was used (effective sample size = 228), a power of at least 80% (84%) was still demonstrated to detect as significant a difference between a prevalence of 10% and one of 25%. Therefore, the study is adequately powered to detect any large effects on prevalence.

2.3 Data management

The questionnaire data and the laboratory results data were received in two separate Microsoft Excel (Office 365, 2016) files. Using Stata version 15 (StataCorp, College Station, TX, USA), the files were merged using the unique reference number that was captured in both documents. The data were cleaned using Microsoft Excel and Stata version 15.

A variable to denote *Campylobacter* status of a farm was created using the frequency of *Campylobacter* cases per farm, where a farm with one or more cases was positive and a farm with zero cases was negative. In order to describe the study sample of farms by *Campylobacter* status, sociodemographic variables and basic farm characteristics (type of housing, size of the flock and type of management system) were included.

Farm-level prevalence estimates were estimated using the binary outcome variables, *Campylobacter* status and NT *S. enterica* status that had a positive or negative value. Bird-level prevalence was calculated using numerical outcome variables, total *Campylobacter* positive birds and total NT *S. enterica* positive birds, which took on values from 0 to 30.

The investigation of possible correlates between bacterial prevalence and farm management and biosecurity practices required the *Campylobacter* binary outcome variable and 31 exposure variables of interest from the biosecurity questionnaire. Some exposure variables were created from a combination of two or more variables in the original data. The details of these adjustments are presented in Supplementary A.

2.4 Exposure variables

The exposure variables included in the analysis are presented in four categories. These are socio-demographic, farm management, biosecurity and other. Supplementary B contains additional information about these variables.

Socio-demographic variables:

- Sex of owner
- Education level of owner
- Area
- State support

Farm management variables:

- Target market
- All-in-all-out system
- Flock size
- House structure
- Number of poultry houses
- Stocking density ≤ 15 birds/m^2
- Days rested between cycles
- Age at slaughter or selling in weeks
- Drinking water treatment
- Open water body less than 1 km away
- Water source
- Storeroom
- Number of farm workers
- Feed bags open/spilled
- Dogs/cats around houses
- Antibiotic use
- *Salmonella* vaccination

Biosecurity variables:

- Overalls provided
- Gumboots provided
- Access control
- Foot bath used and replenished at least daily
- Fenced and gated chicken houses
- Bird-proofed houses
- Rodent control
- Hand hygiene
- Clean house with water/soap and disinfectant
- Disinfection of vehicles entering farm

Other variables:

- Season

2.5 Outcome variable

For the inferential analysis, a binary outcome variable based on the laboratory results for *Campylobacter* spp. (positive or negative) and NT *S. enterica* serovars (positive or negative) was used.

2.6 Descriptive analysis

2.6.1 Descriptive statistics of study sample

Characteristics of the small-scale broiler farms were described by *Campylobacter* spp. status (positive or negative). Categorical variables were described using frequency and percent of farms. The median and interquartile range for the non-normally distributed continuous variables were presented.

2.6.2 Prevalence estimates

Prevalence estimates and 95% confidence intervals were calculated using the proportion command in Stata version 15. If one or more broilers from a farm was positive on culture for *Campylobacter* spp. or NT *S. enterica* serovars, the farm was considered as a positive farm. Farm-level prevalence was estimated using the total number of *Campylobacter*-positive farms as the numerator and the total number of farms sampled for *Campylobacter* as the denominator. Bird-level prevalence of *Campylobacter* was calculated using the total number of *Campylobacter*-positive bird samples as the numerator and the total number of birds sampled for bacterial culture as the denominator. The proportion of *C. coli* and *C. jejuni* species was also determined. Since there were no indeterminate results, all laboratory results were used to calculate the prevalence of *Campylobacter*. The same methods were used to calculate the farm-level and bird-level prevalence of NT *S. enterica* serovars. The bird-level prevalence of *Campylobacter* spp. in three areas of Gauteng Province was estimated using the number of *Campylobacter*-positive birds in Germiston, Tshwane and Randfontein respectively as the numerator, and the total number of birds sampled in each area as the denominator.

2.7 Inferential analysis

Inferential analysis was conducted at the bird-level using logistic regression. The data were aggregated by farm; therefore, frequency weights were applied to represent the frequency of *Campylobacter* positive and negative birds per farm. Observations in the dataset were also clustered by farm. This was accounted for using the clustered sandwich estimator (vce (cluster *clustervar*) option [109] in Stata version 15. Using this method, the standard errors allow for intragroup correlation instead of requiring that observations are independent within groups.

2.7.1 Univariable analysis

Univariable analysis to determine the association of the individual exposure variables with the binary outcome variable was conducted using simple logistic regression. All exposure factors with a p-value less than or equal to 0.2 were considered as potentially significant and were eligible for inclusion in the multivariable model.

2.7.2 Multivariable analysis

Using the potentially significant variables identified from the univariable analysis, backward stepwise elimination with a p-value cut-off of 0.1 was used to select the final multivariable model. Variables that were expected to behave as confounders as indicated by literature or through logical reasoning (routine antibiotic use, season [92,110–112] and age at slaughter [92,110–112]) were retained in the model regardless of the p-value in order to account for potential bias. In the final model, those variables with a p-value less than 0.05 were considered significant. Variables with a p-value between 0.05 and 0.1 were considered as marginally significant. A post-regression link test for model specification was applied [113]. The unadjusted (univariable analysis) and adjusted (multivariable analysis) odds ratios together with 95% confidence intervals and p-values for each included predictor variable were reported.

2.8 Ethical considerations

An ethics waiver was obtained from the Animal Research Ethics Committee of the University of the Witwatersrand in December 2020, certificate reference ‘Waiver 04-12-2020-O’ [Appendix C]. Permission was granted by GDARD to access to the primary data used in the

present study [Appendix D]. Personal identifiers were removed prior to accessing the data. Participants of the primary surveillance activities gave written informed consent [Appendix E].

CHAPTER 3 – RESULTS

In this chapter, the characteristics of the small-scale broiler farms sampled, including farm biosecurity and management practices are described. The prevalence estimates for *Campylobacter* spp. and NT *S. enterica* serovars at farm and bird level are then presented. Finally, the exposure variables that correlated with *Campylobacter* spp. colonisation in the univariable and multivariable analyses are reported.

The chapter headings are:

3.1 Characteristics of the study sample

3.1.2 Biosecurity and management practices of the study sample

3.2 *Campylobacter* spp. and non-typhoidal *Salmonella enterica* serovars prevalence estimates

3.3 Logistic regression model

3.3.1 Univariable correlates of *Campylobacter* spp. colonisation

3.3.2 Multivariable correlates of *Campylobacter* spp. colonisation

3.1 Characteristics of the study sample

Descriptive analysis was conducted at farm-level (**Table 3.1**). Among sampled small-scale farms practicing informal poultry production, local trade in live birds was predominant (24/27, 88.9% of *Campylobacter* positive farms and 2/3, 66.7% of non-colonised farms). In the *Campylobacter* positive group, the majority of farm owners were male (16/27, 59.3%) and had attained either further or higher education levels (11/27, 40.7% each). Farms located in Tshwane were positive for *Campylobacter* (10/10, 100%). Farms from which *Campylobacter* spp. were isolated mainly practiced all-in-all-out management (17/27, 63%) and had a smaller median flock size of 900 birds (IQR 235 – 2500) compared to a median of 1000 birds (IQR 1000 – 1200) in *Campylobacter* negative farms. Open-walled and naturally ventilated poultry houses were predominant in both *Campylobacter* positive (26/27, 96.3%) and *Campylobacter* negative farms (3/3, 100%). Most farms were sampled during summer (19/30, 63.3%).

Table 3.1: Description of the study sample, Gauteng Province, 2020-2021

Characteristic		*Campylobacter* negative farms (N=3)		*Campylobacter* positive farms (N=27)		Total sampled farms (N=30)	
		n	%	n	%	n	%
Sex of farm owner	Male	0	0	16	59.3	16	53.3
	Female	3	100	11	40.7	14	46.7
Education level of owner	Declined/unknown	0	0	2	7.4	2	6.7
	Intermediate (grade 4-6)	0	0	1	3.7	1	3.3
	Senior (grade 7-9)	0	0	2	7.4	2	6.7
	Further education (grade 10-12)	1	33.3	11	40.7	12	40
	Higher education (college/tertiary)	2	66.7	11	40.7	13	43.3
Area	Germiston	2	66.7	8	29.6	10	33.3
	Tshwane	0	0	10	37	10	33.3
	Randfontein	1	33.3	9	33.3	10	33.3
State supported farm	No	3	100	21	77.8	24	80
	Yes	0	0	6	22.2	6	20
Target market	Abattoir	1	33.3	2	7.4	3	10
	Live birds	2	66.7	24	88.9	26	86.7
	Informal slaughter	0	0	1	3.70	1	3.3
All-in-all-out management	No	1	33.3	10	37	11	36.7
	Yes	2	66.7	17	63	19	63.3
Flock size	Median [IQR]	1000 [1000-1200]		900 [235-2500]		1000 [300-2400]	
Structure of poultry house	Free range permanently/part of day	0	0	1	3.7	1	3.3
	Open walls, natural ventilation	3	100	26	96.3	29	96.7
Season at sample collection	Autumn	0	0	11	40.7	11	36.7
	Summer	3	100	16	59.3	19	63.3

3.1.2 Biosecurity and management practices of the study sample

Biosecurity measures to prevent the introduction and spread of pathogens were inconsistently implemented in the small-scale broiler farms (Table 3.2). Treatment of drinking water (such as chlorination) was not practiced in the majority of farms (25/27, 93% of *Campylobacter* positive farms). Just over half of all farms utilised borehole water. In most *Campylobacter* positive farms, feed, cleaning equipment and disinfectant were stored separately. Spillage of feed bags occurred in a third of colonised poultry farms and the presence of dogs or cats near to the poultry houses was common. Antibiotic use was common across the study sample. The provision of gumboots and overalls was inconsistent in both *Campylobacter* positive and *Campylobacter* free farms. Use of footbaths and the regular change of disinfectant used in the footbaths were poorly implemented. Rodent and bird control methods were not used in the majority of *Campylobacter* positive farms. Few farms practiced hand hygiene and disinfection of vehicles entering the farm. It was also evident that 48% (13/27) of farms had poor poultry house cleaning and disinfection practices.

Table 3.2: Biosecurity and farm management exposure variables and *Campylobacter* status of 30 small-scale broiler farms, Gauteng Province, 2020-2021

Exposure variable		*Campylobacter* negative farm (N=3)		*Campylobacter* positive farm (N=27)		Total sampled farms (N=30)	
		n	%	n	%	n	%
Number of poultry houses	Median [IQR]	1 [1-1]		1 [1-2]		1 [1-2]	
Stocking density ≤ 15 birds/m^2	No	0	0	5	18.5	5	16.7
	Yes	3	100	22	81.5	25	83.3
Poultry house rest days	Median [IQR]	8 [7-21]		9 [7-14]		8.4 [7-14]	
Age at slaughter/selling (weeks)	4	1	33.3	3	11.1	4	13.3
	5	0	0	8	29.6	8	26.7
	6	2	66.7	13	48.1	15	50
	7	0	0	3	11.1	3	10
Drinking water treatment	No	3	100	25	93	28	93.3
	Yes	0	0	2	7	2	6.7
Open water body ≤ 1km from farm	No	3	100	20	74	23	76.7
	Yes	0	0	7	26	7	23.3
Water source	Borehole	1	33.3	15	55.6	16	53.3
	Municipal	2	66.7	12	44.4	14	46.7
Storeroom	None	0	0	9	33.3	9	30
	Separate for feed, cleaning equipment and disinfectant	1	33.3	11	40.7	12	40
	Single storeroom	2	66.7	7	25.9	9	30
Number of farm workers	Median [IQR]	3 [2-3]		2 [1-4]		2[2-4]	
Feed bags open/spilled	No	3	100	19	70.4	22	73.3
	Yes	0	0	8	29.6	8	26.7

Exposure variable		*Campylobacter* negative farm (N=3)		*Campylobacter* positive farm (N=27)		Total sampled farms (N=30)	
		n	%	n	%	n	%
Dogs/cats around poultry house	No	0	0	11	40.8	11	36.7
	Yes	3	100	16	59.2	19	63.3
Antibiotic use	No	0	0	6	22.2	6	20
	Yes	3	100	21	77.7	24	80
***Salmonella* vaccination**	No	3	100	26	96.3	29	96.7
	Yes	0	0	1	3.7	1	3.3
Overalls provided	No	1	33.3	16	59.2	17	56.7
	Yes	2	66.7	11	40.8	13	43.3
Gumboots provided	No	2	66.7	10	37	12	40
	Yes	1	33.3	17	63	18	60
Access control	No	1	33.3	14	51.9	15	50
	Yes	2	66.7	13	48.1	15	50
Footbath used and disinfectant changed daily	No	0	0	14	51.9	14	46.7
	Yes	3	100	13	48.1	16	53.3
Fenced farm and gated poultry house	No	0	0	12	44.4	12	40
	Yes	3	100	15	55.5	18	60
Bird-proofed poultry houses	No	1	33.3	16	59.2	17	56.7
	Yes	2	66.7	11	40.8	13	43.3
Rodent control	No	1	33.3	13	48.1	14	46.7
	Yes	2	66.7	14	51.9	16	53.3
Hand hygiene	No	2	66.7	18	66.7	20	66.7
	Yes	1	33.3	9	33.3	10	33.3
Clean house with water/soap and disinfectant	No	2	66.7	11	40.8	13	43.3
	Yes	1	33.3	16	59.2	17	56.7
Disinfection of vehicles entering farm	No	2	66.7	25	93	27	90
	Yes	1	33.3	2	7	3	10

3.2 *Campylobacter* and non-typhoidal *Salmonella enterica* prevalence estimates

The prevalence of *Campylobacter* spp. at farm-level was high (27/30, 90%), and half of the broilers sampled were positive for *Campylobacter* on culture (448/900, 49.8%). Prevalence ranged widely between three areas of Gauteng Province (59/300, 19.7% to 214/300, 71.3%). The dominant *Campylobacter* species isolated was *C. coli* (316/448, 70.5%). No NT *S. enterica* serovars or any other *Salmonella* serovar were cultured from the broiler samples (Table 3.3). The 95% confidence interval suggests that a prevalence of up to 17.2% at farm-level and 4.0% at bird-level was possible.

Table 3.3: *Campylobacter* spp. and non-typhoidal *S. enterica* prevalence estimates, Gauteng Province, 2020-2021

***Campylobacter* spp.**		**n/N**	**Prevalence estimate (%)**	**95% confidence interval**
Farm-level		27/30	90	73.4 – 97.9
Bird-level	Any *Campylobacter* spp.	448/900	49.8	46.5 – 53.1
	C. coli	316/448*	70.5	66.1 – 74.7
	C. jejuni	167/448*	37.3	32.8 – 41.9
	Germiston area	59/300	19.7	15.3 – 24.6
	Tshwane area	175/300	58.3	52.5 – 64.0
	Randfontein area	214/300	71.3	65.9 – 76.4
Non-typhoidal *S. enterica* serovars		**n/N**	**Prevalence estimate (%)**	**95% confidence interval**
Farm-level		0/30	0	0.0 – 17.2
Bird-level		0/900	0	0.0 – 4.0

*Prevalence estimates for *C. coli* and *C. jejuni* include 35 samples that were co-infected with both *Campylobacter* species.

3.3 Logistic regression model

3.3.1 Univariable correlates of *Campylobacter* colonisation

Inferential analysis was conducted at bird-level, using 900 poultry observations. Findings from the univariable analyses of all exposure variables are tabulated (Table 3.4). The demographic variable for education level of the owner was eligible for inclusion in the multivariable model. Variables relating to farm management that were potentially significant were state support of the farm and poultry house structure. Biosecurity related exposures that were considered for inclusion in the multivariable model were: the presence of an open water body less than one kilometre from the farm, treatment of drinking water, feed spillage or open feed bags, the provision of gumboots and the application of rodent control measures. The summer season was also an exposure of potential significance.

Table 3.4: Univariable logistic regression findings of possible exposures correlated to *Campylobacter* spp. positivity, Gauteng Province, 2020 - 2021

Variable		**% *Campylobacter* positive, n/N**	**Unadjusted OR (95% confidence interval)**	**p value**
Education level of owner	Declined/unknown	18.3%, 11/60	Reference	
	Intermediate (grade 4-6)	90%, 27/30	40.10 (13.37 – 120.25)	**<0.001**
	Senior (grade 7-9)	46.7%, 28/60	3.90 (0.60 – 25.23)	**0.153**
	Further education (grade 10-12)	58.9%, 212/360	6.38 (1.43 – 28.55)	**0.015**
	Higher education (college/tertiary)	43.6%, 170/390	3.44 (0.91 – 13.02)	**0.069**
State support	Yes (vs. no)	17.8%, 32/180	3.52 (0.81 – 15.34)	**0.094**
All-in-all-out	Yes (vs. no)	48.8%, 278/570	0.90 (0.29 – 2.76)	0.848

Variable		% Campylobacter positive (n/N)	Unadjusted OR (95% confidence interval)	p value
Poultry house structure	Open walls, natural ventilation (vs. free range permanently/ part of the day)	51.3%, 446/870	14.73 (8.41 – 25.78)	**<0.001**
Flock size			1.00 (1.00 – 1.00)	0.810
Poultry house rest days			1.00 (0.92 – 1.08)	0.941
Open water body ≤ 1km from farm	Yes (vs. no)	76.7%, 161/210	4.61 (1.14 – 18.75)	**0.033**
Water source	Borehole (vs. municipal)	57.3%, 275/480	1.92 (0.63 – 5.82)	0.252
Storeroom	None	61.9%, 167/270	Reference	
	Separate for feed, cleaning equipment and disinfectant	48.9%, 176/360	0.59 (0.16 – 2.18)	0.428
	Single storeroom	38.9%, 105/270	0.39 (0.09 – 1.75)	0.221
Stocking density ≤ 15 birds/m^2	Yes (vs. no)	47.1%, 353/750	0.51 (0.12 – 2.27)	0.380
Access control	Yes (vs. no)	46%, 207/450	0.74 (0.24 – 2.24)	0.592
Number of poultry houses			1.09 (0.79 – 1.51)	0.589
Age at slaughter/selling (weeks)			1.01 (0.52 – 1.93)	0.983
Drinking water treatment	Yes (vs. no)	96.7%, 58/60	33.46 (7.01– 159.71)	**<0.001**
Footbath used and disinfectant changed daily	Yes (vs. no)	49%, 235/480	0.93 (0.31 – 2.81)	0.901
Dogs/cats around poultry house	Yes (vs. no)	53%, 302/570	1.42 (0.45 – 4.48)	0.549
Fenced farm and gated poultry house	Yes (vs. no)	43.3%, 234/540	0.52 (0.17 – 1.59)	0.253
Hand hygiene	Yes (vs. no)	42.3%, 127/300	0.64 (0.19 – 2.16)	0.470
Number of farm workers			1.15 (0.89 – 1.47)	0.287
Bird-proofed poultry houses	Yes (vs. no)	47.2%, 184/390	0.83 (0.27 – 2.54)	0.747
Antibiotic use	Yes (vs. no)	51.4%, 370720	1.38 (0.45 – 4.25)	0.572
Feed bags open/spilled	Yes (vs. no)	68.8%, 165/240	2.93 (0.82 – 10.49)	**0.098**
Gumboots provided	Yes (vs. no)	58.1%, 314/540	2.34 (0.74 – 7.47)	**0.150**
Overalls provided	Yes (vs. no)	56.3%, 219/390	1.57 (0.50 – 4.92)	0.437
Rodent control	Yes (vs. no)	39.2%, 188/480	0.40 (0.13 – 1.20)	**0.101**
Season at sample collection	Summer (vs autumn)	34.4%, 196/570	0.16 (0.05 – 0.49)	**0.001**
Clean house with water/soap and disinfectant	Yes (vs. no)	52.7%, 269/510	1.32 (0.44 – 3.98)	0.627
Disinfection of vehicles entering farm	Yes (vs. no)	36.7%, 33/90	0.55 (0.10 – 2.91)	0.483

3.3.2 Multivariable correlates of *Campylobacter* colonisation

The unadjusted and adjusted odd ratios of variables correlated with *Campylobacter* positivity in the final multivariable model are presented (Table 3.5). The odds of *Campylobacter* colonisation among broilers that received treated drinking water was 86.81 times the odds of colonisation for broilers drinking untreated water. *Campylobacter* spp. positivity among broilers reared in an open-walled poultry house structure was 3.82 times as likely compared to broilers reared in free-range production systems. The odds of *Campylobacter* positivity where feed bag spillage occurred was 12.48 times the odds of positivity where feed bags were sealed. The summer season had a protective effect, with 94% reduced odds of colonisation compared to the autumn season. The following two variables were not statistically significant since the p values were greater than 0.05 and the 95% confidence intervals included the value of 1. Antibiotic use appeared to decrease the odds of C*ampylobacter* positivity by 60%, but this exposure was only marginally significant (p value = 0.088). Lastly, for every one-week increase in the age of broilers at slaughter compared to four weeks of age, the odds of isolating *Campylobacter* were reduced by 37% (p value = 0.148). The equation for the final logistic regression model is presented in Supplementary C.

Table 3.5: Variables correlated with *Campylobacter* spp. positivity in the final multivariable model, Gauteng Province, 2020-2021

Variable		Unadjusted OR (95% confidence interval)	p value	Adjusted OR (95% confidence interval)	p value
Poultry house structure	Open walls, natural ventilation (vs. free range permanently/ part of the day)	14.73 (8.41 – 25.78)	<0.001	3.82 (2.09 – 6.97)	**<0.001**
Age at slaughter/selling (weeks)		1.01 (0.52 – 1.93)	0.983	0.63 (0.34 – 1.18)	0.148
Drinking water treatment	Yes (vs. no)	33.46 (7.01 – 159.71)	<0.001	86.81 (4.56 – 1651.50)	**0.003**
Antibiotic use	Yes (vs. no)	1.38 (0.45 – 4.25)	0.572	0.40 (0.14 – 1.15)	0.088
Feed bags open/spilled	Yes (vs. no)	2.93 (0.82 –10.49)	0.098	12.48 (2.88 – 54.12)	**0.001**
Season at sample collection	Summer (vs autumn)	0.16 (0.05 – 0.49)	0.001	0.06 (0.02 – 0.20)	**<0.001**

The linktest to investigate misspecification errors showed that the t-test for hatsq was not significant (p value = 0.869). The linktest provided some evidence that the final model was correctly specified, accounted for all relevant variables and did not include irrelevant variables.

CHAPTER 4 – DISCUSSION, RECOMMENDATIONS AND CONCLUSION

In this chapter, the major prevalence findings for *Campylobacter* spp. and non-typhoidal *S. enterica* serovars and significant biosecurity or farm management correlates are discussed in the context of the available previous literature. This discussion is then followed by the limitations of the study and recommendations for small-scale broiler farmers and consumers, as well as for future research in this field. Finally, the key contributions of this research are presented in the conclusion.

The chapter headings are:

4.1 Key study findings
4.2 Contextualisation of key findings
4.3 Limitations of the study
4.4 Recommendations
4.5 Conclusion

4.1 Key study findings

This cross-sectional study aimed to estimate the prevalence of *Campylobacter* spp. and NT *S. enterica* serovars in small-scale broiler production systems in Gauteng Province. Farm management and biosecurity correlates of these pathogens were also investigated.

A high prevalence of *Campylobacter* spp. in small-scale broiler production systems, dominated by *C. coli*, was determined. Conversely, NT *S. enterica* serovars were not isolated from sampled broilers. Important biosecurity measures such as hand hygiene, water treatment and disinfection of vehicles entering the farm were not practiced by many sampled farms. The multivariable logistic regression model that accounted for clustering of observations by farm, identified three farm management and biosecurity correlates of *Campylobacter* spp. positivity. Open-walled and naturally ventilated poultry house structures compared to free-range production systems was positively correlated with isolation of *Campylobacter* spp. Predictive practices included feed spillage or unsealed feed bags and drinking water treatment. The summer season had a protective effect. Factors that remained in the final model but were not statistically significant included age in weeks at slaughter or selling and the use of antibiotics in the poultry cycle.

4.2 Contextualisation of key findings

The high prevalence of *Campylobacter* spp. in this study sample concurs with a previously estimated 47.1% prevalence in commercial free range chickens in South Africa [55]. However, the same study reported a *Campylobacter* spp. prevalence that was nearly 20% higher (68%) in chickens produced in a rural setting [55]. The authors did not define 'rural farming systems' but these may be comparable to the small-scale production systems sampled in the present study. Therefore, present study results indicate a lower *Campylobacter* spp. prevalence in small-scale broilers than previously demonstrated in South Africa. A declining local prevalence was also supported by an observational study conducted in 2016 in Kwa-Zulu Natal Province that described a 78% *Campylobacter* spp. prevalence in commercial broilers [96]. Conversely, lower *Campylobacter* spp. prevalence estimates (40.1%) were suggested by a recent meta-analysis of African-based prevalence studies [66]. This difference in prevalence may be due to different sample types (caecal contents and faecal swabs versus cloacal swabs) [75], diagnostic test sensitivity and production systems (commercial versus small-scale production systems).

Compared to observational studies that also performed bacterial culture using cloacal swabs, the present study *Campylobacter* spp. prevalence estimate was in the middle-range of both lower (38.1% - 40.5%) [58,59] and substantially higher (71.4%) [79] estimates. Therefore, although substantially higher estimates have been reported, the burden of *Campylobacter* spp. in broilers in Gauteng Province appears to be nearly 10% higher than estimates from other developing regions.

Surprisingly, *C. coli* was isolated more frequently than *C. jejuni.* This is the opposite to multiple studies including meta-analyses, which identified *C. jejuni* as the most prevalent *Campylobacter* spp. [58,60,61,66,67,71]. Indeed, most human campylobacteriosis cases are caused by *C. jejuni* [4,11,12]. A similar finding was reported from Spain, however, samples were collected at slaughterhouses and not at broiler farms [114]. Interestingly, a local study conducted in 2008 - 2009 similarly found that *C. coli* was the dominant species in commercial free-range broilers and broilers produced in a rural setting but not in layers or industrialised broilers [54]. In Europe, *C. jejuni* and *C. coli* exhibit different seasonal peaks, with *C. coli* tending to increase in prevalence in winter and spring seasons [115]. Given that samples in the present study were collected only in summer and autumn, the higher proportion of *C. coli* isolates compared to *C. jejuni* is likely not related to seasonality. Repeated sampling and *Campylobacter* spp. identification throughout the seasons and for multiple years would be necessary to investigate potential seasonal peaks of *Campylobacter* spp. in local broilers.

The high proportion of isolated *C. coli* is an important finding because there is evidence that a greater proportion of *C. coli* isolates than *C. jejuni* isolates are multidrug resistant (resistant to three or more antimicrobial drugs) [114]. For example, resistance to erythromycin, the first-line treatment of campylobacteriosis, is more frequent in *C. coli* than in *C. jejuni* [115]. This unexpected finding suggests that the small-scale poultry production and consumer industry in Gauteng Province may present an AMR risk and warrants further investigation.

The absence of isolated NT *S. enterica* serovars from sampled broilers was a surprising finding. Thomas et al.'s meta-analysis suggested that the overall *Salmonella* prevalence in African poultry samples ranged from 13.4% - 28,5% [66]. The current study presented a bird-level prevalence range of up to 4% as per the 95% confidence interval. However, this estimate is 15.2% - 25.1% lower than previous South African estimates of 19.2% [54] and 29.1% [116]. Notably, these prevalence studies sampled fresh and frozen chicken carcasses in a retail setting [54] and chicken carcasses from informal chicken outlets [116]. In contrast, the present study

sampled live broilers only, before possible contamination at an abattoir, processing plant or retail setting. Due to the use of different sample types and settings, it is challenging to compare this data with the findings. Non-isolation of NT *S. enterica* serovars is consistent with a South African cross-sectional study estimate of 5% overall incidence of *Salmonella* from 180 298 samples from food-producing animals including poultry, animal feed and animal production environments [53]. Although a prevalence statistic specific to broilers was not offered, the research indicated a generally low prevalence of NT *S. enterica* serovars in South African food-producing animals [53]. Furthermore, prevalence studies in Brazil [71] and New Zealand [70] also did not detect NT *S. enterica* serovars in poultry samples, however, these samples were not collected at the farm-level. Although unanticipated, there is some evidence that a low prevalence of NT *S. enterica* serovars in small-scale broilers is plausible.

The predictive effect of feed spillage on *Campylobacter* positivity can be supported by the literature, which implicates contaminated feed as a source of *Campylobacter* [50,117,118]. Given the possibility of contamination of broiler feed, it is reasonable that sealed feedbags and no feed spillage may be protective. However, there is some evidence that feed is not a major source of *Campylobacter*. Agunos et al.'s meta-analysis to determine sources of *Campylobacter* in broilers at the farm demonstrated that the prevalence of *Campylobacter* in feed samples was low (4.2%, 95% CI 2.3-7.6%) [50]. It is plausible that the feed itself is not the source of bacteria, rather, reservoirs of *Campylobacter* spp. such as wild birds or flies [117] may be attracted to open feedbags or spilled feed. These hosts may contaminate the environment and play a role in the transmission of *Campylobacter* spp. to broilers [117]. Poor feed management and associated contamination of the environment appeared to increase the odds of *Campylobacter* spp. colonisation of broilers.

The positive correlation between an open-walled poultry house compared to a free-range facility and *Campylobacter* positivity was a surprising finding. It is plausible that open-walled poultry houses with natural ventilation may present a contaminated environment, especially if hygienic cleaning and disinfection measures are not applied. This finding is supported by Agunos et al.'s systematic review in which a contaminated rearing environment due to poor disinfection, insufficient downtime and nearby broiler flocks was reported to present the greatest risk of colonisation with *Campylobacter* spp. [50]. Present study results dispute Economou et al.'s finding that the difference between the annual incidence of *Campylobacter* spp. in free-range and conventionally-housed broilers was not statistically significant [119]. This finding should be interpreted with caution because only one sampled farm was free-range

and 93.3% (28/30) of free-range broilers were *Campylobacter* negative. In future studies with a greater proportion of free-range farms, the role of open-walled housing can be further investigated.

Counter-intuitively, drinking water treatment was positively correlated with *Campylobacter* positivity. In contrast, multiple studies reported that water treatment was a protective factor for colonisation with *Campylobacter* spp. [50,58,100,102]. This finding is difficult to substantiate. Drinking water treatment was limited to two sampled farms, at which 96.7% of broilers were positive for *Campylobacter* spp. Therefore, this may be a chance finding and additional sampling from farms that practice water treatment may assist in interpretation. Alternatively, one may hypothesise that adequate water treatment using an appropriate product at the correct concentration was not properly carried out. Due to the cost of disinfectant products, farmers may be reluctant to use the recommended quantity or farmers may inadvertently dilute the chemicals excessively. Another hypothesis is that contamination of the water source by vectors or faecal matter occurred after water treatment. Chance or poor water treatment procedures may explain this finding.

In the present study, the protective effect of summer on *Campylobacter* colonisation is at odds with European studies that report a high prevalence summer season [62,120,121]. There is also scientific evidence that increasing temperatures [121,122], humidity and rainfall [56,123] associated with summer, promote *Campylobacter* spp. transmission. These ecological factors may encourage fly or other biological vector activity [62,120,124] and drive increased survival of *Campylobacter* spp. in the environment leading to contamination of the poultry house [56,123]. In the present study, temperature, rainfall, and humidity data were not collected and could not be analysed. If summer season is considered a proxy for increased fly or vector activity, the summer peak of *Campylobacter* spp. documented in the literature could be explained by transmission from fly vectors to broilers or by fly contamination of the poultry environment. Fly activity and fly-control practices were not assessed at primary data collection. One may hypothesise that increased fly activity in summer prompted small-scale farmers to apply fly control measures. If these measures were effectively used, fly burdens may be controlled and a protective effect of summer and associated use of fly control may be evident.

Although the correlation was not statistically significant, an unexpected negative correlation between increasing age in weeks of broilers at slaughter or selling and *Campylobacter* positivity emerged. This is challenging to explain and differs from a number of studies that

reported increasing age of poultry is associated with an increased risk of colonisation [45,58–60,62,102]. Theoretically, older broilers have a longer exposure period to become colonised with *Campylobacter* [106]. It is also suggested that maternal immunity of broilers at an early age may provide some level of protection [125] and that caecal microflora are resistant to high-level colonization in poultry before 20 days of rearing [126]. In this instance, protective effects of increasing age may be a chance finding or it may have a confounding effect as it does not lie on the causal pathway but is related to disease exposure and *Campylobacter* positivity [102].

Antibiotic use emerged as a marginally significant protective factor in the multivariable model. In agreement with these findings, use of antibiotics in the first two weeks of rearing emerged as a marginally significant negative correlate of *Campylobacter* colonisation (OR=0.33 (95% CI 0.11 – 1.05)) in an Iranian cross-sectional study [60]. Further support is offered by Zbrun et al.'s observational findings. *Campylobacter* was not detected post enrofloxacin antibiotic treatment of broilers, despite the isolation of *Campylobacter* spp. from previous poultry cycles reared in the same poultry house [117]. Assuming that *Campylobacter* spp. isolates were susceptible to the antibiotics administered in small-scale farms, then antibiotic use may have modified the effect of another exposure variable such as feed spillage or poultry house hygiene on the outcome.

Neither of the exposure variables 'open water body less than one kilometre from the farm' nor 'bird-proofed poultry houses' were included in the final multivariable model. This is of interest because the role of contaminated surface water in the epidemiology of *Campylobacter* in animals and humans is well-known, particularly in Europe [40,127,128]. Environmental water contaminated with bacterial agents may act as a source for horizontal transmission to poultry [128]. Attribution studies conducted in Europe reported that a primary contaminator of *Campylobacter* spp. in surface water were wild birds, mainly aquatic species [127,128]. In the present study, detailed data on wild bird activity were not collected. It would be prudent to investigate the possibility of water-borne *Campylobacter* in relation to wild birds and poultry, especially given that only 6.7% of the study sample disinfected the drinking water source.

Poor implementation of certain important biosecurity practices carries implications for numerous avian diseases, including avian influenza [129]. In the context of the small-scale sector, often associated with resource-poor settings, sub-optimal biosecurity practices were expected given the expense of applying some of these measures. Publications assessing the implementation of biosecurity in small-scale poultry farms are scarce [129–131]. An Egyptian

study found that small-scale commercial poultry farmers were reluctant to invest capital into infrastructure needed for biosecurity [130]. In agreement with the present study, the authors also found that hand-hygiene was neglected, and that disinfectant footbath use was not optimal as it often dried out or was incorrectly placed [130]. Cleaning and disinfection protocols did not meet standards and access control was poorly applied by both home-based and farm-based producers [130]. Investigators also recorded discrepancies between survey responses and data collected through participatory methods [130]. This may indicate response bias due to attempts to portray a higher standard of farm biosecurity than is practised. Inadequate biosecurity practices also emerged from a Cameroonian biosecurity survey of small-scale broiler farms [131]. Of the 102 surveyed farms, only 10.8% had a perimeter fencing, 2.9% used dedicated overalls and footwear and 2% disinfected visitors prior to entry [131]. Similar to Egyptian and Cameroonian producers [130,131], sampled small-scale farms lacked important biosecurity features that may be important to transmission and maintenance of *Campylobacter* spp. and NT *S. enterica* serovars in poultry production systems.

4.3 Limitations of the study

While considering the findings of this study, some limitations should be noted. Bacterial culture of *Campylobacter* spp. and *Salmonella* serovars is dependent on specific conditions and growth requirements [132]. Factors such as incorrect handling, transport and storage of sampled specimens or delays in processing at the laboratory may affect the sensitivity of culture diagnostics [132] and lead to an underestimated prevalence of both pathogens. Furthermore, traditional *Campylobacter* culture techniques, such as ISO 10272-1 of 2006 applied in the present study are restricted since it cannot detect viable but non-culturable cells (VBNC). *Campylobacter* spp. are known to enter into a VBNC state after exposure to environmental stressors [133]. Due to this limitation of bacterial culture methods, the presence of *Campylobacter* spp. may be under-estimated. Testing bias may also have influenced the prevalence findings for *Campylobacter* spp. as PCR tests were applied only to culture positive samples. If all cloacal samples were screened using PCR tests it is presumed that a higher *Campylobacter* spp. prevalence would have been estimated. Another factor contributing to underestimation of NT *S. enterica* serovars is the use of bacterial culture tests rather than PCR methods. Greater sensitivity of PCR diagnostics as opposed to traditional bacterial culture for detection of *Salmonella* was established in multiple publications [134–137]. Therefore, the

absence of NT *S. enterica* serovars in the sample group may be an under-estimate due to the use of less sensitive diagnostic techniques.

Epidemiological limitations of this study included the cross-sectional study design which cannot be used to determine causality. A further limitation is that due to the clustering of observations by farm and the reduced effective sample size, the study was powered only to detect a large effect on prevalence, whereas lesser effects may be missed. A methodological limitation occurred because the exposure variables were recorded at the farm level while the outcome was reported at bird level. This limitation was accounted for in the analysis phase by using the clustered sandwich estimator (vce (cluster *clustervar*) option [109] in Stata version 15. In addition, this study was limited to the small-scale broiler sector in Gauteng Province and the study sample was not representative of all types of production systems and regions. Therefore, extrapolation of these findings to other poultry production systems or other provinces in South Africa may not be appropriate. Another epidemiological limitation was selection bias of the small-scale farms approached for data collection. These were randomly selected from a complete list of such farms known to GVS. Presumably, broiler farms attended to by GVS may have more advanced disease control measures in place than farms operating without veterinary guidance. Alternatively, farmers who were known to GVS may have required veterinary intervention due to poor broiler health and high disease burdens.

Despite the intention to investigate correlates of NT *S. enterica* serovars, it was not possible to achieve this objective. Since no NT *S. enterica* isolates were detected in the study sample, regression analysis to identify possible correlates could not be conducted. Since colonisation of broilers with NT *S. enterica* serovars compromises food safety, it would still be valuable to investigate risk factors for colonisation with NT *S. enterica* serovars, possibly in other regions of the country or if NT *S. enterica* serovars are isolated in future routine disease surveillance. Correlation between the exposure variable '*Salmonella* vaccination' and *Campylobacter* positivity could also not be investigated. The exposure factor '*Salmonella* vaccination' was omitted from inferential analyses because all 30 broilers on the single farm that used this vaccine tested positive for *Campylobacter* spp. Therefore, there was no comparison group of *Campylobacter* spp. negative and *Salmonella* vaccinated broilers available in the dataset for analysis.

Primary data collection using a questionnaire relied on self-reporting from the sampled farmers. Despite data collectors' efforts to verify responses through visual inspections during primary

data collection, response bias was likely also a limitation of the present study data. A similar instance of response bias in which discrepancies were observed between survey data and data collected from direct observation was reported in an Egyptian quantitative biosecurity survey [130]. Some examples of factors which may have been affected by response bias and would be difficult to verify visually include level of education, number of poultry house rest days, antibiotic use, and methods of footbath use. If these data were recorded inaccurately, significant correlations between these factors and *Campylobacter* positivity were possibly misidentified.

Due to the secondary nature of this study, certain limitations emerged. Since some important exposure information was not included in the questionnaire during data collection, these factors could not be analysed in the present study. Examples of these exposure factors include: the presence of wild birds around the farm, fly burden and fly control, rainfall, temperature and humidity data, years of farming experience, previous biosecurity training, or past isolation of *Campylobacter* spp. or NT *S. enterica* serovars on the farm. This data would have enriched the analysis and provided greater insight into the role of biosecurity measures and the farm environment in the dynamic between broilers and *Campylobacter* spp.

Lastly, although this study presented prevalence estimates for two important foodborne pathogens at the farm-level, these findings are not a representation of the prevalence of these agents at the abattoir, processing facility or in the retail chain. A host of other factors including protocols followed at the abattoir, hygiene and food safety standard operating procedures at processing plants and in the retail environment will contribute to the final foodborne disease risk presented to the consumer by poultry meat products.

4.4 Recommendations

Based on the study findings and in the context of the small-scale broiler sector, the following recommendations are proposed. A case-control study should follow in order to further investigate the epidemiology of *Campylobacter* spp. and NT *S. enterica* serovars and specifically the risk factors associated with bacterial colonisation in the small-scale broiler sector. This would assist to confirm or rule out the correlates identified in this research. For the purpose of assessing the biosecurity practices of the small-scale broiler sector, formal quantitative evaluations using systematic methods should be carried out. Use of a validated quantitative method such as the Biocheck.UGent scoring system as used in the Philippines [138] or another customised quantifiable tool is advised.

Future prevalence studies should consider the use of PCR tests instead of bacterial culture. The cost differential may be accounted for by using pooled samples. Increased sensitivity of PCR tests would reduce under-estimation of bacterial prevalence that is associated with traditional culture methods for *Campylobacter* spp. and *Salmonella* serovars [77,78,134–137].

In the long term, small-scale broiler farmers should receive biosecurity training that is practical, sustainable and affordable. Special attention should be paid to the risk factors identified in this study, namely feed management, water sanitation and poultry house hygiene. Feed should be sourced from suppliers who follow good agricultural practices and should be kept free from faecal or any other contamination within the poultry house [139]. Feed should be stored in a sealable container with a lid [140]. Regular water quality testing and water decontamination with appropriate products used according the manufacturer's instructions should be promoted [140]. Monitoring of the coliform count in the drinking water is advisable as elevated coliform levels can indicate contamination with enteric pathogens including *Campylobacter* spp. Although coliform count is occasionally only weakly associated with the presence of *Campylobacter* spp., it serves as an important water quality monitoring tool [141]. Thorough cleaning and disinfection of poultry houses should be carried out between broiler flocks [139,140]. Pest and vermin control should be instituted on a continuous basis to prevent contamination of the environment [139]. The biosecurity training sessions should also address the public health threat posed by AMR given the predominance of *C. coli* detected in the study sample. Farmers, with the aid of state and private veterinarians, should be encouraged to collect and submit pooled faecal samples or cloacal swabs for laboratory testing for surveillance of these important foodborne pathogens. If farmers are made aware of the presence of the bacterium in their flocks, they may be more likely to improve disease prevention and control efforts.

Both producers and consumers of poultry products in this informal market sector should be targeted for community-based education programmes on good farming practices and safe food handling and preparation. Hand hygiene should be promoted, especially after contact with broiler faecal matter and litter. Informal slaughter of broilers for home consumption or to be sold to the local community should be discouraged. Since the majority of farms traded locally in live birds, food safety promotion for this market sector should include the principle of avoiding faecal contamination of the poultry carcass during slaughter and thorough cooking of poultry meat. These measures should protect farmers, farm workers and consumers from

salmonellosis, campylobacteriosis and other foodborne pathogens associated with poultry such as *Escherichia coli* and *Listeria monocytogenes* [142].

4.5 Conclusion

In conclusion, this study established that sampled small-scale broiler production systems in Gauteng Province were heavily contaminated by *Campylobacter* spp., while NT *S. enterica* serovars were not highly prevalent in this setting. Furthermore, biosecurity practices of small-scale farmers were inadequate. Feed storage and management, water sanitation and poultry house hygiene emerged as important biosecurity and farm management practices that may reduce the prevalence of *Campylobacter* spp. in small-scale production systems. From a public health perspective, the key contribution of this research was the identification of measures to prevent *Campylobacter* spp. colonisation of broilers produced in small-scale production systems for the promotion of food safety for consumers in this market sector.

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SUPPLEMENTARY MATERIAL

Supplementary A: Manipulations of exposure and outcome variables

Original variable		New variable		Reasoning
Name	**Format**	**Name**	**Format**	
Date of sample collection	String	Season Summer (October-March) Autumn (April-May) Winter (June-August) Spring (September)	Categorical	The month in which sample was collected was used to assign season.
Stocking density	Continuous	Stocking density less than 15 birds/m^2 No Yes	Binary	Grouped based on the accepted stocking density cut-off of 15 birds/m^2
Age at slaughter (days)	Continuous	Age at slaughter (weeks) 4 weeks 5 weeks 6 weeks 7 weeks	Categorical	Number of days divided by seven to calculate weeks.
Presence of water body near farm AND Distance (km) from farm	Binary No Yes Continuous	Open water body less than 1km away No Yes	Binary	Combination of two variables where a water body less than 1 km away was a yes and further than 1km away or no water body nearby at all was a no.
Antibiotic usage	Categorical Never At certain times in the poultry cycle Only when poultry sick	Antibiotic usage No Yes	Binary	Where using antibiotics at certain times in the cycle or when birds are sick was a yes.
Foot bath AND Foot bath change	Binary No Yes Categorical Not daily Do not know Once a day Fresh mix when dirty during the day	Foot bath used and changed daily No Yes	Binary	Combination of two variables were the use of a footbath and changing the disinfectant daily or when dirty during the day was a yes and not using a foot bath or using a foot bath but changing the disinfectant not daily or at unknown frequency was a no.

Fence around houses AND Gate at chicken house	Binary No Yes Binary No Yes	Fenced and gated chicken houses No Yes	Binary	Combination of two variables where the presence of a gate and a fence was a yes and the presence of only a gate or a fence was a no.
Original variable		**New variable**		**Reasoning**
Name	**Format**	**Name**	**Format**	
Cleaning programme	Categorical None Dry clean with broom Wet clean with water Wet clean with water and soap Disinfection after clean	Cleaning and disinfection of house No Yes	Binary	Where wet clean with soap and water and disinfection was a yes and any other answer/combination of answers was a no.
Campylobacter cases	Continuous	*Campylobacter* status Negative Positive	Binary	Where *Campylobacter* cases equal to or greater than 1 was positive and 0 was negative.
Campylobacter cases	Continuous	*Campylobacter* prevalence	Continuous	Where the number of *Campylobacter* cases divided by 30 gave the *Campylobacter* prevalence.

Supplementary B: Exposure variables of interest in the analysis

	Variable	Type	Possible Values
SOCIO-DEMOGRAPHIC	Sex of owner	Binary	Male, female
	Education level of owner	Categorical	None, foundation, intermediate, senior, further education, higher education, declined/unknown
	Area	Categorical	Tshwane, Germiston, Randfontein
	State support	Binary	No, yes
MANAGEMENT	Target market	Categorical	Abattoir, live birds, informal slaughter
	All-in-all-out system	Binary	No, yes
	Flock size	Continuous	Any numerical value
	House structure	Binary	Free range permanently/part of day, open walls, natural ventilation
	Number of poultry houses	Continuous	Any numerical value
	Stocking density ≤ 15 birds/m^2	Binary	No, yes
	Days rested between cycles	Continuous	Any numerical value
	Age at slaughter/selling in weeks	Ordinal	4 weeks, 5 weeks, 6 weeks, 7 weeks
	Drinking water treatment	Binary	No, yes
	Open water body less than 1 km away	Binary	No, yes
	Water source	Binary	Municipal, borehole
	Storeroom	Categorical	None, separate for feed, cleaning equipment and disinfectant, single for feed, cleaning equipment and disinfectant
	Number of farm workers	Continuous	Any numerical value
	Feed bags open/spilled	Binary	No, yes
	Cats/dogs around houses	Binary	No, yes
	Antibiotic use	Binary	No, yes
	Salmonella vaccination	Binary	No, yes
BIOSECURITY	Overalls provided	Binary	No, yes
	Gumboots provided	Binary	No, yes
	Access control	Binary	No, yes
	Foot baths	Binary	No, yes
	Fenced and gated chicken houses	Binary	No, yes
	Bird-proofed houses	Binary	No, yes
	Rodent control	Binary	No, yes
	Hand hygiene	Binary	No, yes
	Clean house with water and soap and disinfect after cleaning	Binary	No, yes
	Disinfection of vehicles entering farm	Binary	No, yes
OTHER	Season	Binary	Summer, autumn

Supplementary C: Final logistic regression model equation

$$\ln[Probability(Campylobacter\ positivity)] = \frac{e^{(4.5+\beta_1(age\ at\ slaughter)+\beta_2(season)+\beta_3(antibiotic\ use\ )+\beta_4(poultry\ house\ structure)+\beta_5(feed\ bags)+\beta_6(water\ treatment))}}{1+\ e^{(4.5+\beta_1(age\ at\ slaughter)+\beta_2 season)+\beta_3(antibiotic\ use\ )+\beta_4(poultry\ house\ structure)+\beta_5(feed\ bags)+\beta_6(water\ treatment))}}$$

Where:

$\beta_1 = 0$	for 4-week age at slaughter
$\beta_1 = 0.63$	for each one-week increase in age at slaughter greater than 4 weeks
$\beta_2 = 0$	for autumn
$\beta_2 = 0.06$	for summer
$\beta_3 = 0$	for not using antibiotics
$\beta_3 = 0.40$	for antibiotic use
$\beta_4 = 0$	for free range permanently/ part of the day
$\beta_4 = 3.82$	for open walled natural ventilated houses
$\beta_5 = 0$	for no feed bags open/spilled
$\beta_5 = 12.48$	for feed bags open/spilled
$\beta_6 = 0$	for no drinking water treatment
$\beta_6 = 86.81$	for drinking water treatment

APPENDICES

Appendix A: Plagiarism declaration

I, Shira Rebeka Amar (student number: 670132), am a student registered for the degree of MSc (Epidemiology) in the academic year 2021/2022.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" software indicating the level of plagiarism in my research document.

Signature 

Date: 23/11/2021

Appendix B: Turnitin cover page

670132:Research_report_SA_TII.docx

ORIGINALITY REPORT

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Signed by:Liesl De Boni
Signed at:2021-11-24 10:17:15 +02:00
Reason:Witnessing Liesl De Boni

Amaya Moll
24 January 2022.

Appendix C: Ethics waiver

ANIMAL RESEARCH ETHICS COMMITTEE
Registration number: AREC-101210-002
Date: 04/12/2020

Certificate reference: Waiver 04-12-2020-O
Category: O
Applicant: Shira Amar (670132**)**
Department: Epidemiology & Biostatistics - National Institute for Communicable Diseases **Tel:** 0761356746; **Email**: shira.amar@wits.ac.za

Re: Waiver from the Animal Ethics Research Committee of the University of the Witwatersrand

This letter is to confirm that Shira Amar (670132), Epidemiology & Biostatistics - National Institute for Communicable Diseases (WITS), does not require full Animal Ethics Research Committee clearance to undertake the work titled **'Prevalence and correlates of Campylobacter and Salmonella species in small-scale broiler poultry, Gauteng, South Africa, 2020-2021'**.

Reason for waiver

This study will not involve specimen collection from animals. Rather, secondary data will be analysed.

Details of the study

The data for analysis are operational data collected by the Gauteng Department of Agriculture and Rural Development (GDARD). Data collection by GDARD is independent of this study. GDARD collects samples from broilers on small-scale poultry farms as part of their disease surveillance activities. The specimens undergo microbial culture testing, and the results of these tests is included in the data that will be used in the research project.

The individuals covered by the waiver are A Shira Amar (Epidemiology & Biostatistics - National Institute for Communicable Diseases).

Please contact me should you require further information.

Yours sincerely

Prof Frederic Michel
Chair: Animal Research Ethics Committee
University of the Witwatersrand

Appendix D: Letter of permission to use data



Umnotho house, 56 Eloff Street, Johannesburg
PO Box 8769, Johannesburg, 2000
Tel: 011 240 2500
Fax: 011 240 2700

Enquiries Dr Liesl De Boni
Telephone 011 240 2695 / 072 997 2218

MEMORANDUM

LETTER OF PERMISSION TO USE DATA

DATE: 4 September 2020

TO: Dr Shira Amar
MSc Epidemiology (field epidemiology) student
Wits school of Public Health, Faculty of Health Sciences, the University of the Witwatersrand
SAFETP field placement at the Gauteng Department of Agriculture and Rural Development

FROM: Dr Dietana Nemudzivhadi
Chief director of Veterinary Services
Gauteng Department of Agriculture and Rural Development
Tel: 011 240 2526, mobile: 071 264 3383, email: dietana.nemudzivhadi@gauteng.gov.za

RE : Permission to use data from GOARD

As the Chief Director of Gauteng Veterinary Services at the Gauteng Department of Agriculture and Rural Development (GOARD), I hereby give Shira Amar permission to access and analyse the data collected by GOARD during surveillance of bacterial infections and biosecurity practices in poultry flocks in the province. This permission is expressly for the MSc research report entitled "Prevalence and correlates of Campylobacter and Salmonella species in small-scale broiler poultry, Gauteng, South Africa, 2020". No personal identifiers are present in the data to be provided. The data are collected as part of GDARD's routine surveillance and service provision to the public. Participation is voluntary and individuals give consent to take part. The student has received approval from GOARD in terms of the Promotion of Access to Information Act, Act No. 2 of 2000. When publishing results forthcoming from analysis of this data, acknowledgment of GOARD and co-authorship of staff involved with the student's study are required.

.....

Dr Dietana Nemudzivhadi

Chief Director: Gauteng Vet Services

Appendix E: Farmer consent form

CONSENT/WAIVER



Surveillance for *Salmonella* and *Campylobacter* and evaluation of biosecurity practices at Gauteng broiler farms

Introduction:

Hello, my name is _____. I am from the Gauteng Department of Agriculture and Rural Development (GDARD). We are interested in assessing the biosecurity practices at your farm. In so doing, we can identify possible areas for improvement which can improve your production. At the same time, we are conducting surveillance for two bacterial diseases that occur in chickens. These are caused by *Salmonella* and *Campylobacter* bacteria.

With your permission, I will ask you several questions about the biosecurity practices on your farm. This may include walking around the premises to confirm the answers. I would also like to collect samples from the birds, to send away for testing. The whole process may take about 2 hours and there is no cost involved for you or your employees.

We will share the results of the testing with you and give you a summary report of the questionnaire that you answered. This will include a summary of the areas for possible improvement on your farm.

Declaration by client:

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I understand that my participation is voluntary and accept that the information gathered at my farm might one day be used for research purposes.

Address: _____

Phone Number: _____

SA Identity number / passport number: _____

Signature: _____ Date (Day/Month/Year): _____

Appendix F: Poultry biosecurity and management questionnaire

Emerging broiler farmer
Surveillance
PoultryBSAudit

Reference Number:

_____

Farm/Owner
Owner full name:

_____

Owner phone number:

_____

Owner gender:

- Male
- Female
- Declined/unknown

Owner education level:

- None
- Foundation (grade 1-3)
- Intermediate (grade 4-6)
- Senior (grade 7-9)
- Further education (grade 10-12)
- Higher education (college/tertiary)
- Declined/unknown

Farm name:

_____

Farm location:

_____

Municipality:

- City of Johannesburg
- City of Tshwane
- Ekurhuleni
- Emfuleni
- Lesedi
- Merafong City
- Midvaal
- Mogale City
- Rand West City

FARM RECORDS
Total birds (flock size):

_____

List: house no., #birds, age, dimension of house (sq metre):

_____

_____

Recent/abnormal mortalities:

- Yes
- No

If yes, how many?

_____

If yes, what signs were noted?

_____

Sick birds currently?

- Yes
- No

If yes to above, what signs were noted?

_____

What is the average % mortality per cycle?

_____

GENERAL MANAGEMENT PRACTICES

Name of company supplying chicks:

_____

Province that chicks come from:

- Eastern Cape
- Free State
- Gauteng
- KwaZulu-Natal
- Limpopo
- Mpumalanga
- North West
- Northern Cape
- Western Cape
- Unknown

Weighing birds:

- By scale
- Judged by eye
- Not done

Target market:

- Live birds
- Abattoir
- Own consumption
- Other

At what age (in weeks) are the birds slaughtered?

_____

What vaccine/s do you use regularly?

- Avivac ND
- Nobilis ND Clone 30
- Hipraviar S
- AviPro ND
- ND La Sota + IBMM

Do you vaccinate against *Salmonella*?

- Yes
- No

How are the birds vaccinated?

- Drinking water
- Spray
- Injection

If water is used, do you:

- ☐ Remove drinking water for a period before vaccinating
- ☐ De-activate Cl if municipal water
- ☐ Use correct dose for No. birds
- ☐ Ensure drank within 1.5 hour
- ☐ Use clean container for mixing
- ☐ Correct No. vials for birds vaccinated
- ☐ Maintain the cold chain while transporting and storage
- ☐ Follow the manufacturer's instructions for diluting

Do you keep feed consumption records?

- Yes
- No

Have you noticed a drop in feed consumption recently?

- Yes

- o No

Date of latest borehole water test:

_____

What were the water test results?

- ☐ *Salmonella* detected
- ☐ *E. coli* detected
- ☐ Neither detected
- ☐ Do not know

Is the drinking water treated?

- o No
- o UV treated
- o Chlorinated
- o Other

Name of feed supplier:

_____

Is the feed tested/treated for Salmonella?

- o Tested for Salmonella
- o Treated for Salmonella
- o Neither
- o Not done

ACCESS CONTROL

Is there a fence around the chicken houses?

- o Yes
- o No

Is the fence….?

- o Intact
- o Effective
- o Maintained

Is there a gate at the entrance to the chicken houses?

- o Yes
- o No

Is the gate locked at all times?

- o Yes
- o No

How many workers enter the farm daily on average?

_____

The farm workers stay:

- o On the farm
- o Off the farm

Do the following vehicles enter the fenced area?

- ☐ Feed trucks
- ☐ Buyers trucks
- ☐ Chick delivery
- ☐ Mortality removal truck/trailer
- ☐ Other

For what purpose do clients/visitors enter the houses?

- ☐ Select chickens
- ☐ Help catch chickens
- ☐ No visitors allowed
- ☐ Other

Are the vehicles that do enter disinfected?

- o Yes
- o No

HOUSING

Is the farm setup by:

- o Government
- o Owner

Is the farm situated close to an open water body (e.g., vlei, dam, wetland)?

- Yes
- No

If yes, how many metres away from the water is the farm?

_____

Type of structure:

- Free range permanently/part of day
- Open walls, natural ventilation
- Closed, environmentally controlled

Is the farm?

- Multi-age
- All-in-all-out

Are the housing floors easy to clean (permanent, e.g., concrete, and not cracked)?

- Yes
- No

What is the stocking density per house (birds/sq metre)?

_____

If curtains are used, are they…

- ☐ In good condition
- ☐ Correctly used
- ☐ Correctly installed

Which of the following biosecurity measures are in place?

- ☐ Double boot system
- ☐ Foot baths
- ☐ Access control
- ☐ Rodent control
- ☐ Bird proof houses
- ☐ Hand hygiene

Do you see rodents in/outside the houses?

- Yes
- No

Method of rodent control

- ☐ Traps
- ☐ Cats
- ☐ Owls
- ☐ Bait stations
- ☐ Other

Specify rodent control method if other:

_____

If you use bait for rodent control, do you alternate the products?

- Yes
- No

Specify fly control product/method:

_____

Storeroom:

- ☐ None
- ☐ Single for feed, cleaning equipment, disinfectants etc.
- ☐ Separate storerooms

Are there open bags or spilled feed lying on the floor at the farm?

- Yes
- No

CLEANING AND DISINFECTION

When do you clean and disinfect?

- During the cycle
- Between the cycle
- During & between the cycle
- Never

For how many days are houses rested between cycles?

_____

What cleaning and disinfection system is used?

- ☐ Dry clean with a broom
- ☐ Wet clean with water
- ☐ Disinfection after clean
- ☐ Wet clean with water and soap
- ☐ None of the above
- ☐ Other

Name the products used for disinfection?

_____

After the client has shown you how they mix the disinfectant, the concentration of disinfectant used is:

- o Correct
- o Inadequate
- o Excessive
- o Do not know

The frequency of mixing disinfectant(s) is done as recommend by the manufacturer

- o Yes
- o No

Litter disposal:

- o Heaped on farm compost
- o Used on vegetable garden
- o Fed to other animals
- o Sold
- o Buried
- o Burned

If litter is heaped on farm/compost, how far from the chicken houses (metres)?

_____

Name of disinfectant used in footbath (if applicable)?

_____

Foot-bath change:

- o Once a day
- o Fresh mix when dirty during the day
- o Just add concentrate during the day
- o Not daily
- o Do not know

Foot-bath concentration:

- o Correct concentration with a measuring jug/device
- o Add until you smell the product
- o Custom measure used

HYGIENE CONTROL

Which of the following are true?

- ☐ Overalls provided by farm
- ☐ Gumboots provided by farm
- ☐ Presence of cats/dogs around houses
- ☐ Litter checked daily
- ☐ Wet patches in litter

Litter material:

- o Saw-dust
- o Shavings
- o Paper
- o Grass
- o Other
- o None

Where is the litter sourced from?

_____

Depth of sawdust/material

- o None
- o 1-3 cm
- o 4-6 cm
- o 7cm and above

Saw-dust management

- o Turning throughout cycle

- o Turn only until third week
- o Never turn
- o Continuously added
- o Remove wet patches

Do wild birds actively feed on disposed material?

- o Yes
- o No

Do workers take care not to carry disposed material back into the houses during daily routines?

- o Yes
- o No

Mortalities are removed

- o Once a day
- o Throughout the day
- o Less often

Mortalities are disposed of:

- ☐ Burying
- ☐ Burning
- ☐ Open disposal on dump
- ☐ Fed to other animals
- ☐ Family consumes
- ☐ Sold

Mortalities are disposed of:

- o On site
- o Off site

If on site, how far from the chicken houses (metres)?

_____

BIRD HEALTH

Do the staff perform daily health checks?

- o Yes
- o No

Health check includes:

- ☐ Mortalities
- ☐ Not eating
- ☐ Ruffled feathers
- ☐ Sneezing/coughing/snucks
- ☐ Diarrhoea
- ☐ Depressed

List the medications used (if applicable)

_____

Do you use…?

- ☐ StressPack
- ☐ PhosPack

Use of Antibiotics (other than in feed)

- o At certain times in cycle
- o Only when sick
- o Every cycle

Name of antibiotic(s) used:

_____

Is the correct withdrawal period correctly managed and adhered to?

- o Yes
- o No

Are you aware that there are diseases that people can catch from sick chickens?

- o Yes
- o No

Other comments:

_____