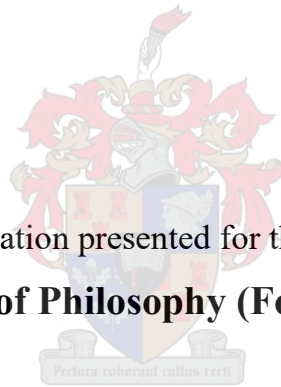# The prevalence of *Campylobacter* and *Arcobacter* species in ostriches from South Africa

by

Nompumelelo Shange

Dissertation presented for the degree of
Doctor of Philosophy (Food Science)

at

Stellenbosch University

Food Science, Faculty of AgriSciences

Supervisor: Prof. P.A. Gouws
Co-supervisor: Prof. L.C. Hoffman

March 2020

DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated) that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March 2020

SUMMARY

The overall aim of this thesis was to determine the prevalence of *Campylobacter* and *Arcobacter* species in ostriches from South Africa. In humans *Campylobacter* and *Arcobacter* species can cause of gastroenteritis, Guillian Barré syndrome, septicaemia and bacteraemia. Previous research has indicated that the consumption of contaminated poultry meat is the main route of infection for humans and by extension poultry species are deemed primary reservoirs of *Campylobacter* and *Arcobacter* species. Currently, there is a lack of information regarding *Campylobacter* and *Arcobacter* species in relation to ostriches from South Africa. Artificially and naturally reared ostrich chicks at the age of 2, 4, 6 and 12 weeks were sacrificed, and caeca samples were excised. *Campylobacter* spp. (*C. jejuni*) was detected in artificially reared chicks, on the 12th week. A persistent presence of *Arcobacter* (*A. skirrowii*) was detected from the 2nd until 12th week of life for both artificially and naturally reared ostrich chicks. Additionally, cohorts that belonged to the same batch as the sacrificed ostrich chicks, regardless of the rearing process were sampled at the slaughter age of 10 and 12 months. *Arcobacter* spp. (*A. skirrowii*) and *Campylobacter* spp. (*C. jejuni*) were isolated from 56-70% of slaughter age birds. Cloacal swabs were also obtained from live ostriches reared on 30 different farms situated in South Africa (Oudtshoorn). Cloacal swabs were processed with family specific PCR (n = 168 pooled cloacal swabs), the Cape Town protocol (n = 836 cloacal swabs), ISO 10272-1:2006 (n = 836 cloacal swabs) and a selective *Arcobacter* spp. method (n = 415 cloacal swabs). Family specific PCR determined an average prevalence of 24.63%. The ISO 10272-1:2006 method and Cape Town Protocol determined a prevalence of 16.83% and 0% for *Campylobacter* spp., respectively. For *Arcobacter* spp. a prevalence of 18.80% and 39.14% was determined with the Cape Town protocol and selective *Arcobacter* spp. method, respectively. Higher prevalence levels were determined when ostriches were sampled during spring and autumn, respectively. Higher prevalence levels were also detected in ostriches reared on farms that made use of borehole water. Higher prevalence levels were seen for ostriches reared on farms with wild water birds. During slaughter, *Arcobacter* spp. were detected at a prevalence level of 73% at post-skinning. At post-evisceration, 73% and 83% of samples were contaminated with *Campylobacter* spp. and *Arcobacter* spp., respectively. At post-chilling, 66% and 67% were contaminated with *Campylobacter* spp. and *Arcobacter* spp., respectively. Additionally, a second study to evaluate the occurrence of *Campylobacter* spp. and *Arcobacter* spp. was conducted to see whether routine testing was required for abattoirs. *E. coli* and coliforms were also enumerated to determine the occurrence of faecal contamination during slaughter. Overall, a low occurrence of *Campylobacter* spp. (0.98% and 0%), *Arcobacter* spp. (1.31% and 1.64%), *E. coli* (0.13 log cfu/g) and coliforms (0.53 log cfu/g) was determined for all three abattoirs. Antibiotic resistance in *Campylobacter* spp. and *Arcobacter* spp. isolated from ostriches and ostrich meat was determined. *Campylobacter* spp. and *Arcobacter* spp. isolates were generally resistant to antibiotics in the following order cephalothin, vancomycin and erythromycin and tetracycline. The majority of *Campylobacter* spp. (92.86%) and *Arcobacter* spp. (80.95%) isolates exhibited multi-drug resistance.

Overall, this research shows that ostriches from South Africa can be considered as potential carriers of species belonging to the *Campylobacteraceae* family and infection can occur at young age. Carcasses can

be contaminated during slaughter and species carried by ostriches can be resistant to essential antibiotics; ultimately highlighting the need for routine testing of *Campylobacter* and *Arcobacter* species.

OPSOMMING

Die algehele doel van hierdie tesis was om die voorkoms van *Campylobacter* en *Arcobacter* spesies in Suid Afrikaanse volstruise te bepaal. *Campylobacter* en *Arcobacter* spesies kan diarree, Guilian Barré sindroom, septisemi, en bakteriemieën in mense veroorsaak. Vorige navorsing het getoon dat die verbruik van besmette pluimveevleis die belangrikste roete van besmetting is vir mense. Pluimveevleis word sodoende gesien as primêre reservoir vir *Campylobacter* spp. en *Arcobacter* spp. Daar is tans n tekort aan inligting rakende *Cam*pylobacter en *Arcobacter* spesies met volstruisvleis van Suid Afrika. Volstruiskuikens wat kunsmatig of natuurlik grootgemaak is teen die ouderdomme van 2, 4, 6, en 12 weke oud opgeoffer en derm monsters geneem. *Campylobacter* spesies (*C. jejuni*) is gevind in kuikens wat kunsmatig grootgemaak is op 12 weke ouderdom. 'n Teenwoordigheid van Arcobacter (*A. skirrowii*) was gevind vanaf die 2de tot en met die 12de week van ouderdom in beide die kunsmatig en natuurlik groot gemaakte kuikens. Monsters is ook geneem van groepe volstruiskuikens wat aan dieselfde groep as die opgeofferde behoort het op 10 en 12 maande ouderdom, ongeag van die grootmaak metode. Voorkomste van *Arcobacter* spp. (*A. skirrowii*) en *Campylobacter* spp. (*C. jejuni*) op vlakke van 56-70% was gevind. Deppers van die kloaka van lewende volstruise op 30 verskillende plase in Suid Afrika (Oudtshoorn) was geneem. Kloaka deppers was geprosesseer deur 'n familie-spesifieke PCR (n =138 saamgestelde deppers), die Kaapstadse protokol (n = 836 deppers), ISO 10272-1:2006 (n = 836 deppers), en 'n selektiewe metode vir *Arcobater* spp. (n = 415 deppers). Familie-spesifieke PCR het 'n gemiddelde voorkomste van 24.63% vasgestel. Die ISO 10272-1:2006 metode en Kaapstadse protokol het 'n voorkomste van 16.83% en 0% onderskeidelik vasgestel vir *Campylobacter* spp. 'n Voorkomste van 18.80% en 39.14% onderskeidelik was vasgestel vir die Kaapstadse protokol en die selektiewe *Arcobacter* spp. metode. Hoer vlakke van voorkoms was vasgestel in volstruise gedurende lente en herfs onderskeidelik. Hoër voorkomste was ook bepaal op plase wat gebruik maak van boorgat water en met wilde voëls. *Arcobacter* spp. was gevind gedurende slagting in 73% van gevalle na afslagting. Na ontweiding was 73% en 83% van die vleis monsters besmet met *Campylobacter* spp. en *Arcobacter* spp., onderskeidelik. Bykomend is 'n studie gedoen om die voorkoms van *Campylobacter* spp. en *Arcobacter* spp. te evalueer om te bepaal of gereelde toetsing by slagpale 'n vereiste moet wees. Gedurende hierdie toetse was die voorkoms van *E. coli* kolonies ook getel om vas te stel of fekale besmetting wel voorkom. Die voorkoms van *Campylobacter* spp. (0.98% en 0%), *Arcobacter* spp. (1.31% en 1.64%), *E. coli* (0.13 log cfu/g) en kolivorme (0.53 log cfu/g) was teenwoordig in al drie slagpale. Bestandheid teen antibiotika in *Campylobacter* spp. en *Arcobacter* spp. isolate van volstruise en volstruisvleis was ook bepaal. Isolate van *Campylobacter* spp. en *Arcobacter* spp. was oor die algemeen bestand teen antibiotika in die volgende orde: cephalothin, vancomycin en erythromycin and tetracycline. Die meerderheid van die isolate van *Campylobacter* spp. (92.86%) en *Arcobacter* spp. (80.95%) het meervoudige dwelmweerstandigheid getoon.

Hierdie navorsing wys dat vostruise van Suid Afrika beskou kan word as 'n moontlike draer van spesies wat aan die *Campylobacteraceae* familie behoort en dat besmetting op 'n jong ouderdom kan plaasvind. Karkasse mag ook besmet word gedurende die slagproses en die spesies wat gedra word deur volstruise kan

bestand wees teen noodsaaklike antibiotika, en beklemtoon dus die noodsaaklikheid van gereelde toetsing vir *Campylobacter* en *Arcobacter* spesies in volstruisvleis.

PREFACE

This dissertation is presented as a compilation of seven chapters. Each chapter is introduced separately and is written according to the style of the journal *International Journal of Food Science and Technology* to which Chapter two was submitted for publication and Chapter 3 was presented at a symposium.

Chapter 1	**General Introduction**
Chapter 2	**Literature review**
	Campylobacter and *Arcobacter* species in food-producing animals*
Chapter 3	**Research results**
	The onset of *Campylobacter* spp. and *Arcobacter* spp. colonisation in ostriches from South Africa*
Chapter 4	**Research results**
	The prevalence of *Campylobacter* and *Arcobacter* species in ostriches from Oudtshoorn, South Africa*
Chapter 5	**Research results**
	Prevalence of *Campylobacter* and *Arcobacter* species on ostrich carcasses during processing
Chapter 6	**Research results**
	Antibiotic resistance patterns of *Campylobacter* spp. and *Arcobacter* spp. isolates obtained from ostriches and ostrich meat from the Western Cape, South Africa
Chapter 7	**General discussion, conclusions and recommendations**

*Partially published as: Shange, N., Gouws, P. & Hoffman, L.C. (2019). *Campylobacter* and *Arcobacter* species in food-producing animals: prevalence at primary production and during slaughter. *World Journal of Microbiology and Biotechnology*, **35**, 146.

*Partially published as: Shange, N., Gouws, P. & Hoffman, L.C. (In-press). The prevalence of *Campylobacter* and *Arcobacter* species in ostriches from Oudtshoorn, South Africa. *Journal of Food Protection.*

*Presented as: Shange, N., Gouws, P.A. & Hoffman, L.C. (2019). The onset of *Campylobacter* spp. and *Arcobacter* spp. colonisation in ostriches from South Africa. RMAA conference and congress, Stellenbosch, South Africa.

This dissertation is dedicated to my late father, **Lawrence Shange** and my late brother, **Sifiso Shange**

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

Prof. Gouws, my supervisor, for your unwavering support and providing your technical expertise during the past three years.

Prof. Hoffman, my co-supervisor, for your consistent belief in my capabilities. Thank you for your encouragement, especially during the difficult times. Thank you for pushing me beyond my limitations and supporting my academic dreams since 2013.

The Archibald Mafeje Scholarship for Advanced study (Tiso Foundation), for their financial assistance.

Ostrich industry and experts within the industry, for taking my calls, helping with sampling, granting access to slaughter facilities when needed and understanding the importance of the study. Also, thank you for your interest in the study.

Fellow post-graduate students and staff members from the Food and Animal Sciences departments, especially, **Elizabeth Sivhute**, for your words of encouragement, **Efaishe Kavela**; my partner in the lab, **Dr. Michaela van den Honert** for your help with PCR, **Veronique Human** for your hugs, **Dr. Rip** for your advice and **Lisa Uys** for your open door.

My siblings and my mother, for never doubting my decision to resign from my job and pursue another research degree. Thank you for the support and prayers. I'm blessed to have sisters like **Lindiwe Shange, Lungile Shange, Philisiwe Augustine** and **Nikiwe Ndawonde** and a mother like **Fikile Shange**. This one is for us girls!

Roderick Juba, my husband, thank you for being an important part of my support system, encouraging me and imparting your wisdom when needed. Thank you for always listening and uplifting me. I am blessed to be loved by you.

Thank you **Heavenly father** for hearing every single prayer. I am here right now because you willed it so.

'*Now all glory to God, who is able, through his mighty power at work within us, to accomplish infinitely more than we might ask or think*'.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
bp	Base pair
CBA	Columbia Blood Agar
CDC	Centre for Disease Prevention and Control
CDT	cytolethal distending toxin
Ceph	Cephalosporin
CFU	Colony Forming Unit
Cip	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CTP	Cape Town Protocol
DAFF	Department of Agriculture, Forestry and Fisheries
DALY	Disability Adjusted Life Years
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EFSA	European Food Safety Authority
Ery	Erythromycin
ETEC	Enterotoxigenic *Escherichia coli*
EU	European Union
FERG	Foodborne Disease Burden Epidemiology Reference Group
Fig.	Figure
FSANZ	Food Standards Australia New Zealand
GBS	Gillian Barré syndrome
h	Hour
ICMSF	International Commission on Microbiological Specification for Food
ISO	International Organization for Standardization
mCCDA	modified Charcoal Cefoperazone Deoxycholate Agar
MDR	Multi drug resistance
MERC	Markets and Economic Research Centre
MFS	Miller Fisher Syndrome
MHA	Mueller Hinton Agar
Min	Minute
ml	Millilitres
mm	Millimetres

mM	Millimolar
MPN	Most Probable Number
NA	Nalidixic Acid
ND	Not detected
PCR	Polymerase Chain Reaction
PHO	Public Health Ontario
PUFA	Poly unsaturated fatty acids
R	Resistant
RTE	Ready to Eat
Sec	Second
SANS	South African National Standard
STEC	Shiga toxin *Escherichia coli*
T	Tetracycline
TBA	Tryptose Blood Agar
UK	United Kingdom
USA	United States of America
V	Vancomycin
VPN	Veterinary Procedural Notices
VRBG	Violet red bile agar
XDR	Extensive drug resistance

CHAPTER 1

Introduction

South Africa is deemed the 'undisputed world leader' in providing ostrich products to the world (Markets and Economic Research Centre (MERC), 2010). The consistent success of the ostrich industry, has been attributed to the best breeding stock, established supporting infrastructure, secure market and experts within the industry (Brand & Jordaan, 2011). Due to these factors, the South African ostrich industry successfully supplies the world with ostrich products, such as ostrich skin, feathers and meat. Predominantly, 90% of the ostrich meat produced in South Africa is exported to the European Union (EU) and Switzerland (MERC, 2010). Overall, the average value gained by the ostrich industry through the export of ostrich meat was estimated to be R370 – 530 million per year from 2006 to 2016 (DAFF, 2016). It should be noted that the export of ostrich meat and by extension, the financial success of ostrich industry has been affected by avian influenza outbreaks (occurring in the year 2004 and 2011) and the economic crisis of 2007/8. Additionally, the most recent ban (lifted at the beginning of 2019) on the export of raw ostrich products, induced by the European Union's concern over the monitoring of residues (antibiotics, antimicrobials, growth promoters and pesticides) has affected the ostrich industry (AgriOrbit, 2018). However, despite the financial difficulties experienced by the industry there is still an interest towards ostrich meat, which can be attributed to the change in consumer dietary preference (Alonso-Calleja *et al.*, 2004). With ostrich meat, the health conscious consumer can be provided with an alternative protein that is lean, low in cholesterol, low in lipid content, high in protein and n-3 polyunsaturated fatty acids (PUFA) content when compared to other types of meat such as beef and chicken (Hoffman & Fisher, 2001; Hoffman & Mellet, 2003; Girolami *et al.*, 2003; Cooper & Horbañczuk, 2002).

Previous research in relation to the microbial quality of ostrich meat is mainly focussed on determining indicator microorganisms in ostrich meat and pathogens such as *Escherichia coli* (*E. coli*) and *Salmonella*. This research focus could be attributed to the fact that microbial specifications for these microorganisms are well established, as seen in Veterinary Procedural Notices (VPN) 52/2018. Currently, there is an apparent need for research that is focused on emerging microorganisms that can affect public health such as *Campylobacter* and *Arcobacter* species. *Campylobacter* and *Arcobacter* species are deemed the leading cause of gastrointestinal infections in humans from developed and developing countries. These species have also been implicated in the cause of more severe illnesses such as Gillian Barré syndrome, bacteraemia and septicaemia in humans (Vandenberg *et al.*, 2004; Kaakoush *et al.*, 2015). Infections in humans are mainly induced by the consumption of contaminated food of animal origin, where literature places a special emphasis on the consumption of contaminated poultry meat and poultry meat products (Evers *et al.,* 2008; Elmali & Can, 2016; Skarp *et al.,* 2016). In this regard, poultry species are seen as the primary reservoirs whilst other food-producing animals such as cattle, sheep and goat are seen as a secondary reservoirs (Shange *et al.*, 2019). This in turn, indicates that poultry species are successfully colonised at primary production possibly reaching prevalence levels ranging from 6 to 100%, as seen for broiler chickens, geese, ducks, pheasants and ostriches (Shange *et al.*, 2019). Colonisation could occur at primary production due to the fact that *Campylobacter* and *Arcobacter* species are ubiquitous in the farm environment and through horizontal transmission they can

2

asymptomatically infect a flock/herd. Horizontal transmission is aided by transmission vehicle/vectors such pets, flies, insects, farm equipment, farm workers, transport vehicles, litter, pests, rodents and wild migratory birds (Shange *et al.*, 2019). Additionally, when a free-range farming system is the focus, previous research has indicated that constant access to the environment can aid in the onset of an infection and possibly result in higher prevalence levels in free range animals (Heuer *et al.*, 2001). Furthermore, as reviewed by Shange *et al.* (2019), previous research has noted that colonisation or an onset of asymptomatic infection can occur after the first 2-3 weeks of life, and once colonisation has occurred an infection can prevail until slaughter age in food-producing animals. Therefore, it could be postulated that horizontal transmission could take place during the rearing of young and older ostriches at primary production, but with only a few prevalence studies and a lack of longitudinal studies, this cannot be conclusively stated.

Research to evaluate the slaughter process in relation to the transmission of species belonging to the *Campylobacteraceae* family has shown that certain steps such skinning and evisceration can aid the contamination of carcasses (sterile flesh) with *Campylobacter* and *Arcobacter* species through faecal contamination (Shange *et al.* 2019). Also, deboning can possibly help spread bacterial contamination (Gouws *et al.* 2017a). Even though studies that evaluate the ostrich process in relation to species belonging to the *Campylobacteraceae* family are lacking, the possibility of faecal contamination occurring during the slaughter of ostriches has been proven (Karama *et al.,* 2003). Faecal contamination is a major contributor to *Campylobacter* spp. and *Arcobacter* spp. being present on carcass surfaces as these species are found in the gastrointestinal tract of food producing animals (Shange *et al.* 2019). Karama *et al.* (2003) found that faecal cross contamination occurred during skinning and evisceration; which was attributed to hands previously in contact with the skin contacting carcass flesh. The prevalence of carcasses contaminated with faecal matter was highest during evisceration, even though a reason was not given by Karama *et al.* (2003), it could be postulated that the cause was the rupture of the viscera and/or spillage of intestinal fluid during evisceration (Gouws *et al.* 2017b).

Infections in humans induced by *Campylobacter* and *Arcobacter* species are usually self-limiting and typically do not require treatment. However, severe cases have been reported, whereby patients suffer from prolonged and persistent symptoms (Kaakoush *et al.*, 2015; Banting & Figueras, 2017). In severe cases the natural treatment progression is the use of antibiotics. Therefore, food that is contaminated with antibiotic resistant bacteria is a major public health concern (van den Honert *et al.*, 2018). In this regard, when food-producing animals harbour antibiotic resistant bacteria and in turn the contamination of meat products with *Campylobacter* and *Arcobacter* species which are resistant to essential antibiotics (used to treat infections in human) is also an important consideration at primary production and during slaughter.

Due to the dearth of information regarding species belonging to the *Campylobacteraceae* family in relation to ostriches from South Africa, the overall aim of this dissertation was to determine the prevalence of *Campylobacter* and *Arcobacter* species in ostriches from South Africa. This was achieved by firstly determining the onset of *Campylobacter* and *Arcobacter* species in ostrich chicks (Chapter 3). Secondly, the prevalence of *Campylobacter* and *Arcobacter* species in ostriches reared in the Oudtshoorn region (Western Cape, South Africa) was determined (Chapter 4). Additionally, the effect of slaughter operations on the

contamination of ostrich carcasses with *Campylobacter* spp. and *Arcobacter* spp. was investigated (Chapter 5). Furthermore, since antibiotic resistance in important foodborne pathogens is a growing area of concern and the contamination of ostrich products with antibiotic resistant *Campylobacter* spp. and *Arcobacter* spp. can affect public health, it was necessary to determine antibiotic resistance patterns of *Campylobacter* spp. and *Arcobacter* spp. isolated from ostriches and ostrich meat (Chapter 6).

1.1 References

AgriOrbit. (2018). The ostrich leather industry: Going from strength to strength. [Internet document]. URL https://www.agriorbit.com/the-ostrich-leather-industry-going-from-strength-to-strength/ 15/08/2019.

Alonso-Calleja, C., Martínez-Fernández, B., Prieto, M. & Capita, R. (2004). Microbiological quality of vacuum-packed retail ostrich meat in Spain. *Food Microbiology*, **21**, 241–246.

Banting, G. & Figueras, S. M. J. (2017). Global Water Pathogen Project. [Internet document]. URL http://www.waterpathogens.org/book/*Arcobacter* 08/08/2018.

Brand, T.S. & Jordaan, J.W. (2011). The contribution of the South African ostrich industry to the national economy. *Applied animal husbandry & rural development*, **4**, 1–7.

Cooper, R.G. & Horbañczuk, J.O. (2002). Anatomical and physiological characteristics of ostrich its nutritional importance for man. *Animal Science Journal*, **73**, 167–173.

Department of Agriculture, Forestry and Fisheries (DAFF) (2016). A profile of the South African ostrich market value chain. [Internet document]. URL http://www.nda.agric.za/doaDev/sideMenu/Marketing/Annual%20Publications/Commodity%20Profiles/Livestock/Ostrich%20market%20value%20chain%20profile%202015.pdf. 02/10/2017.

Elmali, M. & Can, H.Y. (2016). Occurence and antimicrobial resistance of *Arcobacter* species in food and slaughterhouse samples. *Food Science and Technology (Campinas)*, **37**, 280–285.

Evers, E.G., van der Fels-Klerx, H.J., Nauta, M.J., Schijven J.F. & Havelaar, A.H. (2008). *Campylobacter* source attribution by exposure assessment. *International Journal of Risk Assessment and Management*, **8**, 174–189.

Girolami, A., Marsic, I. D'Andrea, G., Braghieri, A., Napolitano, F. & Cifuni., G.F. (2003). Fatty acid profile, cholesterol content and tenderness of ostrich meat as influenced by age at slaughter and muscle type. *Meat Science*, **64**, 309-315.

Gouws, P.A., Shange, N., & Hoffman, L.C. (2017a). Microbial quality of springbok (*Antidorcas marsupialis*) meat in relation to harvesting and production process. In: *Game meat hygiene: Food safety and security* (edited by P. Paulsen, A, Bauer, & F.J.M. Smulders). Pp. 223-228. Netherlands: Wageningen Academic Publishers

Gouws, P.A., Shange, N., & Hoffman, L.C. (2017b). The microbial quality of black wildebeest (*Connochaetes gnou*) carcasses processed. In: *Game meat hygiene: Food safety and security* (edited by P. Paulsen, A, Bauer, & F.J.M. Smulders). Pp. 229-234. Netherlands: Wageningen Academic Publishers.

Heuer, O.E., KPedersen, JSAndersen & MMadsen. (2001). Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. *Letters in Applied Microbiology*, **33**, 269–274.

Hoffman, L. C. & Mellett, F. D. (2003). Quality characteristics of low-fat ostrich meat patties formulated with either pork lard or modified corn starch, soya isolate and water. *Meat Science*. **65**, 869–75.

Hoffman, L.C. & Fisher, P. (2001). Comparison of meat quality characteristics between young and old ostriches. *Meat Science*, **59**, 335–337.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, **28**, 687–720.

Karama, M., Jesus, A.E. De & Veary, C.M. (2003). Microbial quality of ostrich carcasses produced at an export-approved South African abattoir. *Journal Of Food Protection*, **66**, 878–881.

Markets and Economic Research Centre (MERC) (2010). The South African Ostrich sub-sector. [Internet document]. URL http://www.namc.co.za/upload/all%20reports/Ostrich%20Value%20Chain%20Report.pdf 02/10/2017.

Ramees, T.P., Dhama, K., Karthik, K., Rathore, R.S., Kumar, A., Saminathan, M., Tiwari, R., Malik, Y.S. & Singh, R.K. (2017). *Arcobacter*: An emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control - A comprehensive review. *Veterinary Quarterly*, **37**, 136–161.

Shange, N., Gouws, P. & Hoffman, L.C. (2019). *Campylobacter* and *Arcobacter* species in food - producing animals: prevalence at primary production and during slaughter. *World Journal of Microbiology and Biotechnology*, **35**, 146.

Shirzad Aski, H., Tabatabaei, M., Khoshbakht, R. & Raeisi, M. (2016). Occurrence and antimicrobial resistance of emergent *Arcobacter* spp. isolated from cattle and sheep in Iran. *Comparative Immunology, Microbiology and Infectious Diseases*, **44**, 37–40.

Skarp, C.P.A., Hänninen, M.L. & Rautelin, H.I.K. (2016). Campylobacteriosis: The role of poultry meat. *Clinical Microbiology and Infection*, **22**, 103–109.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N., Zissis, G. & Butzler, J. (2004). *Arcobacter* species in humans. *Emerging Infectious Diseases*, **10**, 1863-1867.

van den Honert, M.S., Gouws, P.A. & Hoffman, L.C. (2018). Importance and implications of antibiotic resistance development in livestock and wildlife farming in South Africa: A Review. South African Journal of Animal Sciences, 48, 401–412.

Veterinary Procedural Notices (VPN) 52 (2018). Guidelines on the verification of compliance with process hygiene control and microbiological food safety performance objectives under the Meat Safety Act. URL http://www.rmaa.co.za/wp-content/uploads/2018/12/VPN-52-Microbiological-Reference-Criteria-for-Meat-19-October-2018-Draft.pdf. 5/08/2019.

CHAPTER 2

Literature review: *Campylobacter* and *Arcobacter* species in food-producing animals[*]

2.1 Introduction

Increasing meat consumption around the world directly correlates with the concern of meat safety. The presence of pathogens can negatively affect meat safety resulting in economic loss and major food losses (Alagić *et al.*, 2016). Certain parts of the world are not ready for pathogenic outbreaks, as seen in South Africa's recent listeriosis outbreak that resulted in over 200 deaths. South Africa's listeriosis outbreak illuminated a bigger problem, which is the need for strengthened food safety and surveillance systems in some parts of the world (especially in developing countries) (Clarke, 2018). Currently, species that belong to the *Campylobacteraceae* family are important pathogens as *Campylobacter* species are deemed as the leading cause of gastroenteritis and the cause of severe illnesses such as Gillian Barré syndrome and reactive arthritis in humans from developed and developing countries (Kaakoush *et al.*, 2015). Similarly, *Arcobacter* species can cause gastroenteritis and more severe diseases such as bacteraemia and septicaemia (Collado & Figueras, 2011). *Campylobacter* and *Arcobacter* species are ubiquitous in nature and also reside in the gastrointestinal tract of many animals including food-producing animals. Food sources, especially those of animal origin have been majorly implicated in the cause of *Campylobacter/Arcobacter* related infections in humans, implying successful colonisation at primary production, contamination during slaughter operations and survival in food products once contamination has taken place. Therefore, within this literature review the typical characteristics of *Campylobacter* and *Arcobacter* species will be presented. Infections caused by *Campylobacter* spp. and *Arcobacter* spp. in humans will be discussed. This literature review will also focus on *Campylobacter* and *Arcobacter* species in animals (with a special focus on food-producing animals) and *Campylobacter* spp. and *Arcobacter* spp. contamination during the slaughter of food-producing animals. Lastly, regulatory information pertaining to *Campylobacter* and *Arcobacter* species in food products will be discussed.

2.2 Species that belong to the *Campylobacteraceae* family

Species that belong to the *Campylobacter* and *Arcobacter* genera belong to the *Campylobacteraceae* family. The *Campylobacteraceae* family also encompasses the genus *Sulfurospirillum* (Lastovica, On & Zhang, 2014), however, this section (and by extension, this literature review) will only outline typical characteristics for species that belong to the *Campylobacter* and *Arcobacter* genera.

---

[*]Partially published as: Shange, N., Gouws, P., Hoffman, L.C. (2019). *Campylobacter* and *Arcobacter* species in food-producing animals: prevalence at primary production and during slaughter. *World Journal of Microbiology and Biotechnology*, **35**, 146.

2.2.1 *Campylobacter* species

Campylobacter species are gram-negative, non-spore forming microorganisms that are micro-aerophilic (5-10 % oxygen and carbon dioxide of 3-5%). Most *Campylobacter* spp. cells have a spiral, curved rod appearance with a width ranging from 0.2 to 0.8 µm and length ranging from 0.5 to 5 µm (Keener *et al.*, 2004). Generally, *Campylobacter* species have a non-sheathed flagellum and move in a fast cork-screw like motion, due to the presence of single or multiple flagella at one or both ends (Keener *et al.*, 2004). The presence of a flagellum (or flagella) allows for movement through the mucous layer of the intestinal tract and also viscous growth media. However, *C. gracilis* and *C. hominis* are non-motile (Keener *et al.*, 2004; Lastovica, 2006). Generally, *Campylobacter* species can grow in temperatures ranging from 30 to 42ºC, with the thermo-tolerant *Campylobacter* species preferring an optimum temperature of 41.5ºC. Thermo-tolerant *Campylobacter* species are not 'true thermophiles' due to their inability to grow at 55ºC. *Campylobacter* species cannot grow at temperatures below 30ºC (Keener *et al.*, 2004), due to an absence of cold shock protein genes, which aid in cold temperature adaptation (Levin, 2007). Furthermore, *Campylobacter* species grow in an environment with the water activity of 0.997 and a pH range of 6.5-7.5. Water activity of ≤0.99, pH of ≤4.9, and a pH of ≥9 can result in growth inhibition of *Campylobacter* species (Keener *et al.*, 2004). Unlike most bacteria, *Campylobacter* species do not catabolise carbohydrates, rather they make use of amino acids with serine, aspartate, asparagine, and glutamate being preferred, in that order. Alternatively, tricarboxylic acid cycle (also referred to as citric acid cycle) intermediates such as 2-oxoglutarate, succinate, fumarate and malate can be used as an energy source (Stahl *et al.*, 2012).

The number of species and subspecies that belong to the *Campylobacter* genus is a subject surrounded by discrepancies and warrants finalisation as researchers have not yet come to a conclusion. For instance, in 2001 16 species and six sub-species were reported (On, 2001), whilst in 2006 17 species and six sub-species were reported (Korczak *et al.*, 2006; Lastovica, 2006) and in 2013, 26 species were reported (Euzeby, 2013). However, the most recent research regarding the taxonomy of the genus *Campylobacter* validly describes 23 species and 12 subspecies phenotypically (colony morphology, optimum growth temperature, atmospheric preference and biochemical properties) as seen in Table 2.1 (On *et al.*, 2017).

Table 2.1 A list of *Campylobacter* species and subspecies that have been validly described by On *et al.* (2017)

Species	Sub species
C. avium	*C. fetus subsp. fetus*
C. coli	*C. fetus subsp testudium*
C. concisus	*C. fetus subsp venerealis*
C. corcagiensis	*C. hyointestinalis subsp. hyointestinalis*
C. cuniculorum	*C. hyointestinalis subsp. lawsonii*
C. curvus	*C. lari subsp. lari*
C. helveticus	*C. pinnipediorum subsp. pinnipediorum*
C. hepaticus	*C. pinnipediorum subsp. caledonicus*
C. hominis	*C. lari subsp. lari*
C. iguaniorum	*C. lari subsp. lari*
C. insulaenigrae	*C. jejuni subsp. jejuni*
C. jejuni	*C. jejuni subsp. doylei*
C. rectus	
C. showae	
C. sputorum	
C. subantarcticus	
C. upsaliensis	
C. ureolyticus	
C. volucris	
C. lanienae	
C. lari	
C. mucosalis *C. ornithocola* *C. peloridis* *C. pinnipediorum*	

2.2.2 *Arcobacter* species

Species that belong to the *Arcobacter* genus exhibit a similar physiology and characteristics as *Campylobacter* species, as they are also gram-negative, non-spore forming, motile (single polar unsheathed flagellum) curved/helical rods with a width and a length of 0.2-0.9 µm and 1-3 µm, respectively. Similarly, to *Campylobacter* species, *Arcobacter* species can grow in a micro-aerophilic environment. However, unlike *Campylobacter* species, *Arcobacter* species can also grow aerobically (with the exception of *Arcobacter anaerophilus* which is an obligate anaerobe) and they tend to have a broader growth temperature range of 15 to 37ºC, with an optimal temperature being 30ºC under micro-aerophilic conditions (Sasi Jyothsna *et al.*, 2013). Additionally, some *Arcobacter* species such as *Arcobacter butzleri* (*A. butzleri*), *Arcobacter defluvii*, *Arcobacter ellisii*, *Arcobacter molluscorum* and *Arcobacter mytili* have shown high temperature tolerance, due to their ability to survive at high temperatures (42ºC) (On *et al.*, 2017). Typically, *Arcobacter* species can grow within a pH range of 5 to 8.5 and prefer water activity of 0.980. *Arcobacter* species can make use of a

respiratory metabolism and instead of catabolising carbohydrates they make use of organic and amino acids as a source of carbon (Ramees *et al.*, 2017).

With regards to the number of *Arcobacter* species, the *Arcobacter* genus seems to tell a story of expansion rather than confusion (Banting & Figueras, 2017), for instance in 2010 the genus comprised of 10 recognised species (Collado & Figueras, 2011), in 2013 the number of recognised species increased to 15 -17 species (Levican *et al.*, 2013; Sasi Jyothsna *et al.*, 2013) and in 2015 20 species were recognised (Levican *et al.*, 2015). Currently, 28 recognised species have been documented, as seen in Table 2.2 (Ramees *et al.*, 2017; Euzeby, 2018; Kim *et al.*, 2018).

Table 2.2 Recognised species that belong to the *Arcobacter* genus (Euzeby, 2018; Ramees *et al.*, 2017; Tanaka *et al.*, 2017)

Species	Species
A. nitrofigilis	*A. venerupissp*
A. anaerophilus	*A. ellisii*
A. aquimarinus	*A. haliotis*
A. bivalviorum	*A. halophilus*
A. butzleri	*A. lanthieri*
A. canalis	*A. lekithochrous*
A. cibarius	*A. marinus*
A. cloacae	*A. molluscorum*
A. cryaerophilus	*A. mytili*
A. defluvii	*A. suis*
A. ebronensis	*A. thereius*
A. pacificus	*Candidatus Arcobacter sulfidicus*
A. skirrowii	*A. acticola*
A. trophiarium	*A. haliotis*

2.3 *Campylobacteraceae* species in humans

2.3.1 *Campylobacter* spp.

Campylobacter spp. related illnesses are a public health concern. *Campylobacter* species are associated with most of the gastrointestinal infections (campylobacteriosis) in developing and industrialised countries (Aboderin, *et al.*, 2002; Humphrey *et al.*, 2007, Mabote *et al.*, 2011). The World Health Organization (WHO) deemed *Campylobacter* species as the most important gastroenteritis causing pathogens and *Campylobacter* species have been gaining attention since 1977 (Kist, 2002).

Campylobacter related illnesses can be induced by the accidental consumption of contaminated vegetables, milk and meat, however, most infections are attributed to the consumption of contaminated undercooked/partially cooked poultry meat and poultry meat products (Nauta *et al.*, 2009; Allain *et al.*, 2014; González *et al.*, 2016; Heredia & García, 2018). In 2003, the Centres for Disease Control and Prevention, reported that 50-70 % of *Campylobacter* related illness in the United States of America, Europe and Australia, were due to the consumption of poultry and poultry products and to date poultry meat has remained the leading

9

cause of campylobacteriosis (Hald *et al.,* 2016; Percivalle *et al.,* 2016). Evidence of the importance of chicken in *Campylobacter* infections in humans was especially seen in 1999 during the dioxin (toxic chemical compounds) crisis in Belgium. The crisis led to a ban on chicken sales, which also resulted in a 40% reduction in campylobacteriosis cases in Belgium. The importance of chicken as a reservoir of *Campylobacter* spp., was again seen when a reduction in *Campylobacter* related illnesses in the Netherlands occurred in 2003, due to the ban on chicken sales and the subsequent decrease in consumption triggered by the avian influenza outbreak in 2003 (Wagenaar *et al.*, 2006).

Furthermore, non-food sources such as contaminated water, infected person to person contact and infected animal to person contact can cause *Campylobacter* related illnesses in humans (Little *et al.,* 2008; Kaakoush *et al.,* 2015; Hald *et al.*, 2016). However, even though other routes (non-food) of infection for *Campylobacter* do exist, non-food sources are rarely reported making the ingestion of contaminated food the main route of infection in developing and developed countries (Public Health Ontario (PHO), 2014) as seen Table 2.3.

Table 2.3 Outbreaks resulting in *Campylobacter* infections and the source of infection

Region	Number of cases	Source	Year	Reference
New Zealand	29	Water	2014	Bartholomew *et al.* (2014)
Canada	225	Mud	2007	Stuart *et al.* (2010)
	43	Other	2010-2011	Gaudreau *et al.* (2013)
Greece	37	Water	2009	Karagiannis *et al.* (2010)
Norway	12	Animal faeces	2009	Møller-Stray *et al.* (2012)
Denmark	45	Water	2010	Gubbels *et al.* (2012)
Switzerland	55	Water	2008	Breitenmoser *et al.* (2011)
Finland	2	Water	2007	Räsänen *et al.* (2010)
	24	Ground water	2011	González *et al.* (2016)

Table 2.3 Outbreaks resulting in *Campylobacter* infections and the source of infection (continued)

Region	Number of cases	Source	Year	Reference
Finland	3	Poultry	2012	González *et al.* (2016)
United Kingdom	10	Mud	2008	Griffiths *et al.* (2010)
	59	Poultry	2009	Wensley & Coole (2013)
	24	Poultry	2010	Inns *et al.* (2010)
	3	Poultry	2011	Abid *et al.* (2013)
Liverpool	11	Poultry	2011	Farmer *et al.* (2012)
Australia	15	Poultry	2012	Parry *et al.* (2012)
Korea	92	Poultry	2009	Yu *et al.* (2010)

Campylobacteriosis cases have mostly been linked to *C. jejuni* (80-90% of illnesses) and *C. coli* (Skarp *et al.*, 2016) and other *Campylobacter* species have also been associated with human illnesses such as periodontitis and septicaemia (Humphrey *et al.*, 2007). Not all *Campylobacter* species have been associated with diseases in humans, as during detection the growth of other *Campylobacter* species can be inhibited by antibiotics used in the selective media. Furthermore, the temperatures, atmosphere and incubation period used can be inappropriate for the growth of all *Campylobacter* species (Lastovica, 2006). Nonetheless, other *Campylobacter* species have been implicated in infections, as Lastovica (2006) proved that the use of a non-selective passive filtration method can allow for the isolation of *Campylobacter concisus* and *Campylobacter upsaliensis* in diarrheic paediatric patients from South Africa.

Even though the ingestion of *Campylobacter* cells can result in a number of illnesses in humans, it is mostly responsible for gastroenteritis, it is believed that *Campylobacter* spp. is the cause of 400 to 500 million gastroenteritis cases worldwide (Heredia & García, 2018). However, it is noteworthy that in a small (but equally important) number of cases, the infection can be more severe, resulting in Guillian Barré Sydrome (GBS). GBS is an autoimmune complication that can impact the peripheral nervous system, causing respiratory and neurological disorders which can lead to death. For instance, in Bangladesh, GBS has a documented frequency of 3.25/100 000 cases in children older than 15 years old. Other severe cases have reported *Campylobacter* related illnesses leading to Miller Fisher syndrome (MFS) and reactive arthritis (Keener *et al.*, 2004; Lastovica, 2006).

Campylobacter cells ranging from 500 to 800 cfu have been generally accepted to be able to cause Campylobacteriosis (Black *et al.*, 1988; Keener *et al.*, 2004). However, an outbreak involving the ingestion of raw beef liver proved that a lower dose of 360 MPN, could possibly result in an infection in healthy individuals (Hara-Kudo & Takatori, 2011). Additionally, the inoculum ingested tends to be directly proportional to the severity of the disease, meaning the higher the ingested inoculum the more intense the illness is in an individual (Allos & Lastovica, 2011). Once *Campylobacter* cells have been ingested an infection can have an incubation period of 2 to 5 days and tends to last for 1 to 10 days (Humphrey *et al.*, 2007). Campylobacteriosis is characterised as a self-limiting gastroenteritis syndrome manifested as abdominal cramps, abdominal pain, nausea, fever and bloody diarrhoea triggered by pathogenic responses i.e. adhesion and invasion of intestinal

cells (Altekruse & Tollefson, 2003; Kaakoush *et al.*, 2015). The adhesion and invasion of intestinal cells is aided by the presence of a flagella (or flagellum), chemokine response, fimbria like filaments and surface proteins that help with attachment and subsequently the invasion of intestinal cells. Once intestinal cells are invaded this can result in cell injury which ultimately manifest as diarrhoea (Allos & Lastovica, 2011). In addition to the ability to adhere and invade intestinal cells, *Campylobacter* spp. can also produce a cytolethal distending toxin (CDT) which inhibits mitosis of eukaryotic cells and ultimately results in cell death (Silva *et al.,* 2011).

2.3.2 Incidences of *Campylobacter* spp. in developed and developing countries

Campylobacteriosis cases have increased in developed countries such as Australia, Europe, North and Central America (Kaakoush *et al.*, 2015; Heredia & García, 2018) and the incidence rate has been reported to range from 1.3 to 197 per a population size of 100 000 (Table 2.4). In 2010, campylobacteriosis was reported to be the 6th most important global burden contributor, *Campylobacter* species were reported as the most important pathogenic hazard in high income countries and the second most important microbiological hazard in the EU and western pacific regions by The Foodborne Disease Burden Epidemiology Reference Group (FERG) (Hald *et al.*, 2016). The most recent report by the CDC and EFSA, made use of data from 26 EU countries and 2 European Economic Area (EEA) countries, reported campylobacteriosis as the leading zoonosis, with 246307 confirmed cases in 2016 (ECDC & EFSA, 2017). In developed countries *Campylobacter* infections are highest among children (<1 years old) and also the elderly people (>65 years old) (Nielsen *et al.*, 2013). Furthermore, Finland's annual epidemiological report (reporting on 2014 data), showed an increase in risk of *Campylobacter* infection in young adults, this was linked to several factors, such as; young people tend to travel, partake in recreational activities and consume high risk foods (Hemsworth & Pizer, 2006; Nakari *et al.*, 2010). Additionally, *Campylobacter* infections are influenced by season, where peaks in *Campylobacter* infections have been reported during the warmer months of the year (González *et al.,* 2016; ECDC & EFSA, 2017)

Epidemiological data from developing African, Asian and middle eastern countries tells a different story when compared to developed countries (Platts-mills & Kosek, 2015), however it should be noted that this data is incomplete (Kaakoush *et al.*, 2015). This unavailability of information from the developing countries is due to the absence of surveillance bodies such as the ECDC and EFSA who enforce mandatory notification for *Campylobacter* cases (Skarp *et al.*, 2016). Despite this, there is research indicating that campylobacteriosis cases do occur in developing countries. Developing countries, mainly African and South East Asian countries, bore almost half of the global burden of *Campylobacter* (Percivalle *et al.*, 2016) and the incidence has been reported to range from 2.8 to 10.2% in developing countries (Table 2.4). Most campylobacteriosis cases in developing countries are endemic and are mostly limited to children and the risk of campylobacteriosis decreases as age increases, it has been hypothesized that a protective immunity from contracting the infection at an early age occurs (Allos & Lastovica, 2011), however, *Campylobacter* spp. can still be isolated from stools of healthy adults (WHO, 2012). In developing Asian countries *Campylobacter* spp. is one of the five most important cause of diarrhoeal diseases in children and in African countries, *Campylobacter* spp. has been reported in asymptomatic healthy adults (WHO, 2012). Unlike developed

countries, seasonal peaks in *Campylobacter* infections are not prominently seen in developing countries. The lack of seasonal influence has been attributed to seasonal changes not being as drastic as developed countries, as only slight climatic changes occur (Kaakoush *et al.*, 2015).

Table 2.4 A compilation of the recent studies regarding incidence rates of campylobacteriosis in developed and developing countries

Region	Incidence	Reference
European countries	1.3 – 197 per 100 000 246 000 confirmed cases	ECDC & EFSA (2015)
Canadian countries	Ontario – 27.6 per 100 000 3781 confirmed cases	PHO, 2014
USA	6.79 to 16.18 per 100 000	Geissler *et al.* (2017)
Japan	1512 per 100 000	Kaakoush *et al.* (2015)
Australia	112 per 100 000	Kaakoush *et al.* (2015)
New Zealand	161 per 100 000	Kaakoush *et al.* (2015)
Asia - Singapore	5% (n = 100 adult patients)	Chau *et al.* (2016)
Africa - Tanzania	9.7% (n = 300 children >5 years old) 9.64% (n = 2340 children) 6.93% (n = 1622 adults)	Deogratias *et al.* (2014) Komba *et al.* (2013)
Kenya	7.3% (n = 10816 children) 7.53 % (n = 953 adults)	Deogratias *et al.* (2014)
Uganda	9.3% (n = 226 children)	Komba *et al.* (2013)
Rwanda	11% (n = 102 children) 4.65% (n = 98 adults)	Komba *et al.* (2013)
Malawi	21% (hospitalised children with and without diarrhoea)	Mason *et al.* (2013)
South Africa - Limpopo	2.8 – 10.2 % (adult patients with gastroenteritis, HIV and gastrointestinal inflammation)	Samie *et al.* (2007)
South Africa - Durban	25-75% (patients with diarrhoea and dysentery)	Shobo *et al.* (2016)
South Africa – Cape Town	40% (Children with diarrhoea)	Lastovica (2006)

For both developing and developed countries the immune-compromised individuals tend to be affected too. Infection in immune-compromised individual has been reported to be more aggressive and more persistent than any other susceptible group of people (Louwen *et al.*, 2012).

2.3.3 *Arcobacter* spp.

Arcobacter species have been deemed a potential pathogen since 1988, when *A. cryaerophilus* was isolated from human stool. The pathogenesis and the public impact of *Arcobacter* spp. has not been extensively researched as studies solely focussed on *Arcobacter* species are lacking (Banting & Figueras, 2017).

Speculatively, lack of research has been attributed to laboratories and by extension, routine microbiological analysis not making use of appropriate culture methods that can promote growth of *Campylobacter* spp. and also other closely related microorganisms (Vandenberg *et al.*, 2004).

The global distribution of *Arcobacter* is currently unknown. Most of the current information available is from research conducted in developed countries, therefore a comparison between developed and developing countries cannot be made. However, one could speculate that the global distribution could be wider than *Campylobacter* species due to their ability to survive and replicate in water (Banting & Figueras, 2017). Despite the lack of research focussed on *Arcobacter* spp., at least five out of the 28 recognised species have brought attention to the *Arcobacter* genus (Prince Milton *et al.*, 2017). For instance, *A. butzleri* is deemed an 'emerging' pathogen world-wide, has been classified by the International Commission on Microbiological Specification for Food (ICMSF) as a hazard to human health and *A. butzleri* is the fourth most commonly isolated *Campylobacter* like organism in Belgium and France (Vandenberg *et al.*, 2004). The potential of *A. butzleri* as a human pathogen is closely followed by *A. cryaerophilus*, *A. skirrowii*, *A. thereius* and *A. trophiarum*; all species part of the *Arcobacter* genus that have varying incidence levels in population studies (Table 2.5). It should be noted that incidence/prevalence levels can also be swayed by the identification method used, for instance the relatively high incidence level of 13% reported by Samie *et al.* (2007) was achieved with polymerase chain reaction (PCR) rather than culture methods. Additionally, similar to other microbial infections, incidence levels can be influenced by certain factors such as age and health status of an individual; such factors can predispose certain individuals to *Arcobacter* infections (Samie *et al.*, 2007).

Table 2.5 A compilation of population studies related to *Arcobacter* cases categorised by region, incidence level and *Arcobacter* species responsible in each case

Region	Incidence rate (%)	Sample information	Arcobacter species	References
Switzerland	1.4	Healthy people	*A. cryaerophilus*	Houf & Stephan (2007)
South Africa	0.4	Diarrhoeic paediatric patients	A. *butzleri*	Lastovica & Roux, (2000)
South Africa (Limpopo)	13	Diarrhoeic patients	A. *butzleri*	Samie *et al.* (2007)
	3	Asymptomatic patients	*A. cryaerophilus* *A. skirrowii* *Arcobacter* spp.	Samie *et al.* (2007)
France	1.2	Patients with diarrhoea	*Arcobacter* spp.	Abdelbaqi *et al.* (2007)
India	1.5	HIV positive patients with diarrhoea and HIV negative patients	*Arcobacter* spp.	Kownhar *et al.* (2007)
	1	HIV negative patients	*Arcobacter* spp.	Kownhar *et al.* (2007)

Table 2.5 A compilation of population studies related to *Arcobacter* spp. cases categorised by region, incidence level and *Arcobacter* species responsible in each case (continued)

Region	Incidence rate (%)	Sample information	*Arcobacter* species	Reference
New Zealand	0.9	Humans with diarrhoea	A. *butzleri* A. *cryaerophilus*	Gifford *et al.* (2012)
Italy	79	Patients with Type 2 diabetes	*Arcobacter* spp.	Fera *et al.* (2010)
	26.2	Non-diabetic individuals	*Arcobacter* spp.	Fera *et al.* (2010)
Turkey	0.3	Patients with acute gastroenteritis	A. *butzleri*	Kayman *et al.* (2012)
Chile	0.7 -1.4	Patients with diarrhoea	A. *butzleri*	Collado *et al.* (2013)
Netherlands	0.4	Patients with infectious diseases	A. *butzleri*	De Boer *et al.* (2013)
USA and EU	8	Travellers with acute diarrhoea after returning from Mexico/Guatemala/ India	A. *butzleri*	Jiang *et al.* (2010)
Belgium	0.05	Patients with gastroenteritis	*Arcobacter* spp.	Vandenberg *et al.* (2004)
	1.31	Patients with gastroenteritis		Prouzet-Mauléon *et al.* (2006)
Canada	45.5	Non-diarrheic patients	*A. butzleri*	Webb *et al.* (2016)
	56.7	Diarrheic patients	*A. butzleri*	Webb *et al.* (2016)
Southern Chile	3.6	Children with diarrhoea	*A. butzleri*	Fernandez *et al.* (2015)
India	2	Patients with diarrhoea	*A. butzleri*	Mohan *et al.* (2014)

On occasion, *Arcobacter* spp. can cause severe diseases in humans and where there is a severe underlying illness, infections caused by *Arcobacter* spp. have been reported to be more progressive. For instance, *A. butzleri* has been linked to severe diarrhoea and bacteraemia in a neonatal patient with liver cirrhosis. Also, *A. cryaerophilus* and *A. skirrowii* have been reported to cause bacteraemia in a uremic patient (Collado & Figueras, 2011). Usually, typical manifestations are similar to symptoms witnessed for campylobacteriosis as they include: abdominal pain, occasional vomiting, fever and chronic diarrhoea (Vandenberg *et al.*, 2004). However unlike diarrhoea caused by *Campylobacter* spp. which tends to be bloody, *Arcobacter* spp. induces a persistent watery diarrhoea (Vandenberg *et al.*, 2004).

Similar to *Campylobacter* spp., transmission routes for *Arcobacter* spp. include contaminated food especially food that originates from animals. Previous research has associated *Arcobacter* spp. infections with

meat (chicken, pork and beef), milk and cheese (Elmali & Can, 2016). Additionally, similar to *Campylobacter* related illnesses, poultry seems to be a frequent source of *Arcobacter* spp. infections in humans, followed by pork and beef (Collado & Figueras, 2011). Contaminated sea food is also a significant source of infections, as traditionally sea food is eaten raw or partially cooked (Collado *et al.*, 2009; Iwamoto *et al.*, 2010). Furthermore, contaminated recreational and drinking water can be an efficient source of *Arcobacter* spp. and can result in infections (Ramees *et al.*, 2017), as several waterborne outbreaks in Slovenia (Kopilovi *et al.*, 2008), Idaho (Rice *et al.*, 1999), Ohio (Fong *et al.*, 2007) and Turkey (Ertas *et al.*, 2010) have been reported. Waterborne *Arcobacter* spp. infection can be aided by *Arcobacter*'s ability to survive in water by forming biofilms and possibly adhering to the inner surfaces of pipes (Ferreira *et al.*, 2013). Furthermore, an outbreak that occurred at an Italian school illuminated the possibility of person to person transmission of *Arcobacter* spp., as it was found that the isolated strains responsible for the infection were phenotypically and genotypically the same (Vandamme *et al.*, 1992). Other routes of transmission also exist, such as animal to person (Petersen *et al.*, 2007).

2.4 *Campylobacter* and *Arcobacter* species in food-producing animals

2.4.1 Pre-slaughter sources of *Campylobacter* and *Arcobacter* species and routes of transmission

Initial colonisation can occur through horizontal and vertical transmission. Horizontal transmission has been widely reported as successful in transferring *Campylobacter* and *Arcobacter* species originating from vectors such as domestic pets, pests (insects, rodents and migratory birds), farm equipment, transport vehicles, feed, farm workers, litter (poultry houses) and water (Keener *et al.*, 2004; Sahin *et al.*, 2015; Umar *et al.*, 2016; Hald *et al.*, 2016; Hassan, 2017) to flocks/herd, as seen in Figure 2.1. The high genetic diversity of isolates isolated from farm animals could indicate multiple sources of *Campylobacter* and *Arcobacter* species at primary production (De Smet *et al.*, 2011a). For instance, it has been shown that rodents and migratory birds carry *Campylobacter* spp. in the gastrointestinal tract and through defaecation can introduce these pathogens to soil, water sources and feed (Colles *et al*., 2008b; Hamidi, 2018). The dynamics of the transmission route are not fully elucidated in literature; nonetheless it is clear that farmed animals can be exposed to strains carried by pests. For instance, Colles *et al.*, (2008b) isolated the same *Campylobacter* genotypes harboured by migratory birds from free range animals. Additionally, once introduced to water, *Arcobacter* spp. has the potential to grow and survive in surface water due to their ability to form biofilms, as reported by Giacometti *et al.* (2015) who proved a persistent prevalence of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* in water troughs located in cattle and sheep farms. In addition to pests, domesticated animals have been reported to carry *Campylobacter* spp. in the gastrointestinal tract, therefore can also introduce *Campylobacter* spp. to the farm environment (Humphrey *et al*., 2007). When *Campylobacter* spp. and *Arcobacter* spp. have been introduced to the environment, personnel can potentially carry these species on their boots and clothing; physically transferring them from one area to the next (Stanley & Jones, 2003; Federighi, 2017). For instance, Van Driessche *et al.* (2004, 2005) found identical *Arcobacter* strains in non-adjacent pig pens; proving the transmission capability of contamination vectors such as farm workers. Lastly, for poultry it has been noted that the presence of other farm animals (multi-species farming) can introduce *Campylobacter* spp. to flocks

(Umar *et al*., 2016). In previous studies, it was shown that *Campylobacter* spp. carried by other livestock such as cattle can successfully colonise the gastrointestinal tract of broiler chickens. The presence of other livestock on a farm can be a point of interest, as this practice results in an increase of hosts that carry species that belong to the *Campylobacter*aceae family, it should also be noted that the direction of transmission is unclear. Nonetheless, Ridley *et al*. (2011) showed a clear interaction between broiler chickens and cattle, as *Campylobacter* strains shared between the hosts were identified. In literature, it is not clear how transmission occurs, but it is postulated that through vectors such as pests and personnel, strains can be transferred from livestock to poultry flocks.
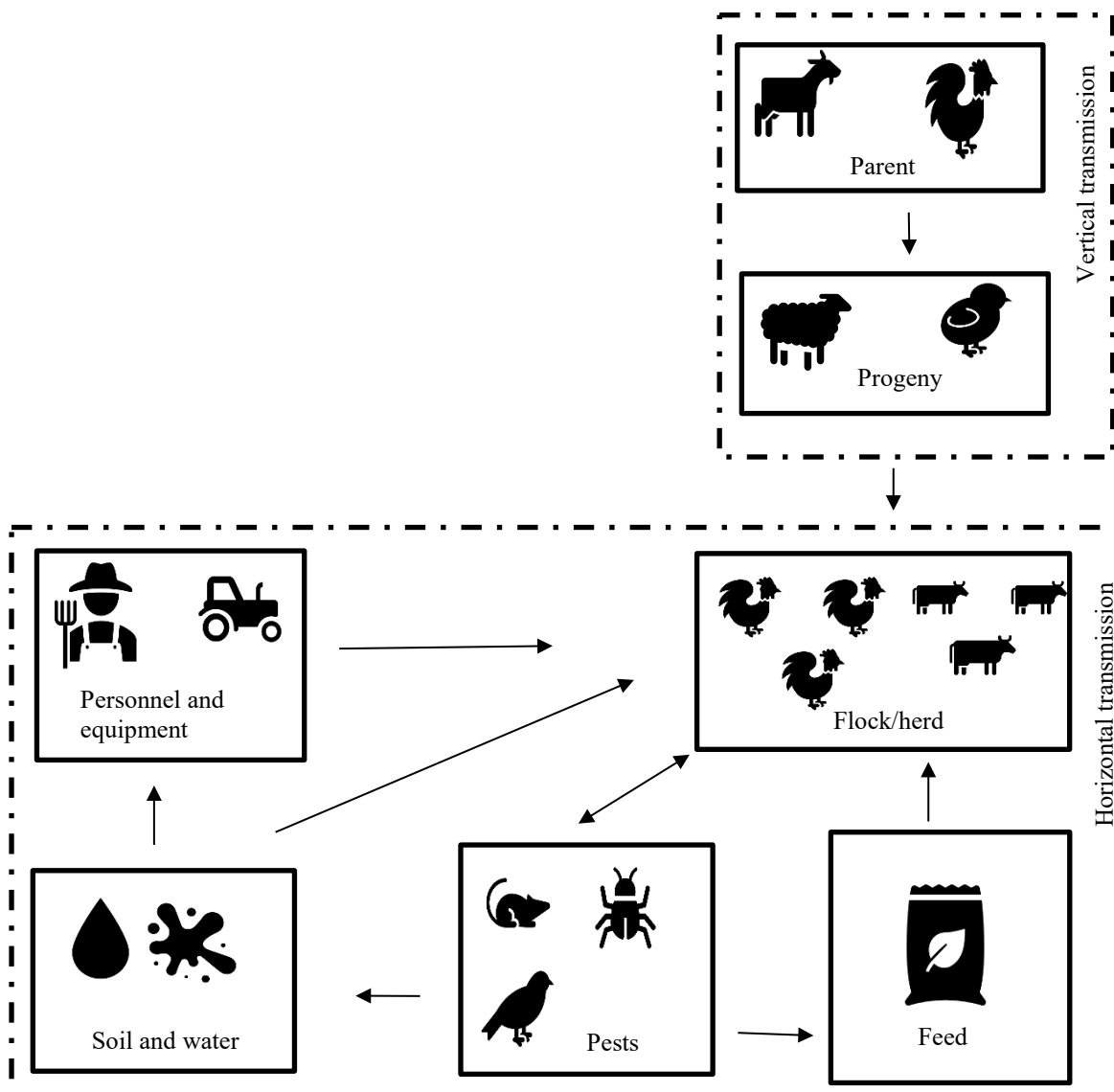
Figure 2.1 Vertical and horizontal transmission of *Campylobacter* spp. and *Arcobacter* spp. to food-producing animals at primary production (Shange *et al.*, 2019).

At primary production vertical transmission of *Campylobacter* and *Arcobacter* species might also occur (Fig. 2.1). However, evidence that supports vertical transmission is scarce. Ho *et al*. (2006) found the occurrence of vertical transmission of *A. cryaerophilus* between sows and their offspring. In this study, *A. cryaerophilus* demonstrated the ability to penetrate the intestine and placenta, resulting in a successful

transmission to the offspring. However, similar evidence has not been presented for cattle, sheep and poultry. In fact, from studying hens and hatchlings, Lipman *et al*. (2008) distinctively proved that breeding hens that carry *Arcobacter* spp. in the gastrointestinal tract and oviduct magnum mucosa could not transmit *Arcobacter* spp. to hatchlings.

For the transmission of *Campylobacter* spp., the same *Campylobacter* strain in breeders has been isolated from their progeny indicating vertical transmission (Callicott *et al*., 2006). *Campylobacter* spp. has also been isolated from semen of roosters, indicating that this could be a possible path of introduction to the breeder hen and subsequently to the inside of the egg and then the chick (Cox *et al*., 2002). However, the possibility of vertical transmission of *Campylobacter* spp. has been reported to be uncommon in poultry as previous research has showed that breeders shedding *C. jejuni* laid eggs that were not infected with *C. jejuni*. However, it has been proposed that faecal matter can contaminate the eggshell resulting in *C. jejuni* permeating through the eggshell and contaminating the inside of the egg (Sahin *et al*., 2003). Similarly, in other food-producing animals such as cattle and sheep, the ability to transmit *Campylobacter* spp. through vertical transmission has been investigated. However, this postulation has been refuted as research has indicated that newly birthed calves and lambs are normally born without *Campylobacter* spp. and only start shedding *Campylobacter* spp. after four days post-partum indicating that they were most likely infected later (Stanley & Jones, 2003).

Once an infection has been established within a flock/herd, initial colonisation can occur within eight days to several weeks, however, the average time is dependent on the number of colony forming units ingested by the animal (Horrocks *et al*., 2009).

2.4.2 Prevalence in poultry

Poultry species have been reported as the primary source of *Campylobacter* spp. and *Arcobacter* spp. infections in developed and developing countries (Kaakoush *et al*., 2015). For *Campylobacter* spp. poultry is the main host possibly due to factors such as high body temperature and the presence of fibronectin. Poultry have high body temperatures ranging from 39 to 41°C, the temperature range is close to *Campylobacter*'s optimal growth temperature of 42°C (Park, 2002; Allos & Lastovica, 2011) and the presence of fibronectin in the gastrointestinal tract of poultry allows for *Campylobacter* spp. to bind through the binding protein CadF (Ziprin *et al*., 1999). Unlike *Campylobacter* spp., for many years it has been unclear whether *Arcobacter* spp. colonised the gastrointestinal tract of poultry as in previous studies researchers could not recover *Arcobacter* spp. from fresh caeca, but could isolate *Arcobacter* spp. in the slaughter environment, this led researchers to suggest water as the potential source of *Arcobacter* spp. during the slaughter of chickens (Van Driessche & Houf, 2007a). However, Atabay *et al*. (2006) has shown that *Arcobacter* spp. do inhabit the gastrointestinal tract of poultry.

Carriage levels of 6 – 9 log cfu/ml can result in detection of *Arcobacter* spp. in poultry (Eifert *et al*., 2003) and *Campylobacter* spp. carriage levels in poultry can be as high as ≥ 4 log cfu/g (Gormley *et al*., 2014) in broiler chickens and 6.7 log cfu/g in pheasants (Seguino & Chintoan-Uta, 2017) at prevalence levels of 6–44% for *Arcobacter* spp. and 2–100% for *Campylobacter* spp. (Table 2.6). Prevalence levels can differ

between studies due to time of sampling, detection methods, and the animal's exposure to environmental sources of *Campylobacter* and *Arcobacter* species (Çelİk *et al*., 2018).

      Currently, diseases induced by *Arcobacter* spp. have not been reported in poultry (Collado & Figueras, 2011) and in most cases poultry are asymptomatic carries of *Campylobacter* species. However, it has been reported that *C. coli*, *C. jejuni* and *Campylobacter lari* could cause gastroenteritis in poultry (Humphrey *et al*., 2007). Furthermore, in one published case, *C. jejuni* and *C. coli* could cause infectious hepatitis in young ostriches (2–8 weeks old) (Stephens *et al*., 1998). Additionally, Humphrey *et al*. (2014) reported that *Campylobacter* spp. can cause inflammation in the gastrointestinal tract of fast-growing broiler chickens.

Table 2.6 Prevalence levels of *Campylobacter* and *Arcobacter* species from chickens, ducks, geese, pheasants and ostriches

Animal	Region	Sample type	Prevalence (%)	Species	Reference
Chickens	Thailand	Caeca samples	11	*C. jejuni*	Chokboonmongkol *et al.* (2013)
	Vietnam	Faecal samples	32	*C. jejuni*	Carrique-Mas *et al.* (2014)
	EU	Faecal samples cloacal swabs	75-100	*Campylobacter* spp.	EFSA (2008)
	Netherlands	Caeca samples	93-97	*C. jejuni* and *C. coli*	Schets *et al.* (2017)
	India	Faecal samples	14.67	*Arcobacter*	Patyal *et al.* (2011)
	India	Faecal samples	8	*Arcobacter*	Mohan *et al.* (2014)
	Upper Egypt	Cloacal swabs	7	*Arcobacter spp.*	Hassan (2017)
		Intestinal samples	44	*Arcobacter* spp.	Hassan (2017)
	Costa Rica	Cloacal swabs	6	*A. butzleri*	Bogantes *et al.* (2015)
		Faecal samples		*A. cryaerophilus*	
Turkeys	Upper Egypt	Cloacal swabs	4	*Arcobacter* spp.	Hassan (2017)
Ducks	Vietnam	Faecal samples	24	*C. jejuni*	Carrique-Mas *et al.* (2014)
	Turkeys	Cloacal swabs	26.14	*Arcobacter* spp.	Çelİk *et al.* (2018)
	Upper Egypt	Cloacal swabs	6	*Arcobacter* spp.	Hassan (2017)
		Intestinal samples	22		

19

Table 2.6 Prevalence levels of *Campylobacter* and *Arcobacter* species from chickens, ducks, geese, pheasants and ostriches (continued)

Animal	Region	Sample type	Prevalence (%)	Species	Reference
Ducks	Costa Rica	Intestinal samples	20	*A. butzleri* *A. cryaerophilus*	Bogantes *et al.* (2015)
Geese	Turkey	Cloacal swabs	100	*C. jejuni*	Aydin *et al.* (2001)
	UK	Cloacal swabs	50	*C. jejuni*	Colles *et al.* (2008a)
	Turkey	Cloacal swabs Faecal samples	16.7 12.93	*Arcobacter spp.*	Çelİk *et al.* (2018)
Geese	Costa Rica	Cloaca swabs faecal samples	10	*A. butzleri* *A. cryaerophilus*	Bogantes *et al.* (2015)
	UK	Cloacal swabs	50	*C. jejuni*	Colles *et al.* (2008a)
Pheasants	Scotland	Cloacal swabs	38	*C. jejuni* and *C. coli*	Seguino & Chintoan-Uta (2017)
	Italy	Cloacal swabs	86.7	*C. coli and C. jejuni*	Dipineto *et al.* (2008)
Partridges	Europe	Cloacal swabs	22-60	*Campylobacter spp.*	Díaz-Sánchez *et al.* (2012)
Ostriches	Alabama	Intestinal swabs	28	*Campylobacter* spp.	Oyarzabal *et al.* (1995)
	Italy	Cloacal swabs	40	*C. jejuni* and *C. coli*	Cuomo *et al.* (2007)
	Malaysia	Cloaca swabs	2	*Campylobacter* spp.	Ling *et al.* (2011)
	India	Faecal samples	6	*Campylobacter* spp.	Prince Milton *et al.* (2017)

2.4.3 Prevalence pigs, cattle and sheep

Pigs have been reported to harbour species that belong to the *Campylobacteraceae* family (Table 2.7) at carriage levels of ≥ 7 log cfu/g for *Campylobacter* spp. (Leblanc Maridor *et al*., 2008; Bratz *et al*., 2013) and ≥ 4 log cfu/g for *Arcobacter* spp. (Van Driessche *et al*., 2004). Generally, *C. coli* and *A. butzleri* are frequently isolated in pigs (Denis *et al*., 2011). However, there is evidence that pigs can carry novel *Arcobacter* species such as *Arcobacter trophiarum* (De Smet *et al*., 2011b) and *Arcobacter cibarius*, as these species have been isolated from pig manure and piggery effluent. Generally, pigs are asymptomatic carriers of *Campylobacter* and *Arcobacter* species, however, it has been reported that *Arcobacter thereius* can cause spontaneous abortion, *Campylobacter mucosalis* can induce necrotic enteritis, *Campylobacter hyoilei* can induce proliferative enteritis, *C. coli, C. jejuni* subsp. *jejuni*, *Campylobacter hyointestinalis* subsp. *hyointestinalis* and *A. butzleri* can cause gastroenteritis in pigs (Humphrey *et al*., 2007; Collado & Figueras, 2011).

Cattle also carry species that belong to the *Campylobacteraceae* family (Table 2.7), at a reported carriage level of ≥ 5.3 log cfu/g for *Campylobacter* spp. (Ramonaitė *et al*., 2013) and 2 log cfu/g for *Arcobacter* spp. (Van Driessche *et al*., 2005). In terms of dominant species in cattle, *C. jejuni* and *C. coli* are associated more with cattle. However, *Campylobacter* species in relation to cattle are not limited to just *C. jejuni* and *C. coli*, as the presence of *Campylobacter doylei*, *Campylobacter fetus* and *Campylobacter hyointestinalis* in faecal matter from healthy cattle from Ghana has been reported (Enokimoto *et al*., 2007). With regards to *Arcobacter* spp., cattle commonly carry *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* (Grove-White *et al*., 2014). Cattle tend to be asymptomatic carriers of species that belong to the *Campylobacteraceae* family but it has been reported that *C. fetus* can cause spontaneous abortion and infectious infertility (Humphrey *et al*., 2007), *A. butzleri* can cause enteritis and *A. skirrowii* can cause diarrhoea as well as haemorrhagic colitis in cattle (Collado & Figueras, 2011).

Sheep have also been associated with *Campylobacter* and *Arcobacter* species (Table 2.7). However, in comparison to pigs and cattle, sheep have not been extensively researched. Nonetheless, sheep have been associated more with *C. coli* and *C. jejuni* (Oporto *et al*., 2007) at carriage levels of 2.9 to 5 log cfu/g (Rotariu *et al*., 2009; Sproston *et al*., 2011). Also, *A. butzleri* and *A. cryaerophilus* have been isolated more frequently in sheep, when compared to other *Arcobacter* species (Shirzad Aski *et al*., 2016). In most cases, sheep are asymptomatic carriers of *Campylobacter* and *Arcobacter* species, but it has been reported that *Campylobacter fetus* subsp. *fetus* can induce spontaneous abortion, *C. coli* can induce gastroenteritis and *A. skirrowii* can cause diarrhoea and haemorrhagic colitis (Humphrey *et al*., 2007; Collado & Figueras, 2011).

Table 2.7 Prevalence of *Campylobacteraceae* species in pigs, cattle, sheep and goats

Animal species	Region	Sample type	Prevalence (%)	Species	Reference
Pigs	Ghana	Faecal samples	29	*Campylobacter* spp.	Karikari *et al.* (2017)
	Japan	Rectal content	42	*C. coli*	Haruna *et al.* (2013)
	Japan	Intestinal swabs	45	*C. coli* and *Campylobacter* spp.	Maramski (2012)
	Nigeria	Faecal samples	92.67	*C. hyointestinalis* *C. lari*	Gwimi *et al.* (2015)
	France and Sweden	Faecal and colon samples	74-77	*C. coli*	Kempf *et al.* (2017)
	Ecuador	Faecal samples	15	*A. cryaerophilus* *A. skirrowii* *A. butzleri* *A. thereius*	Janneth *et al.* (2018)
	India	Faecal samples	21	*Arcobacter* spp.	Patyal *et al.* (2011)
		Faecal samples	12	*Arcobacter* spp.	Mohan *et al.* (2014)
	Southern Chile	Faecal samples	40.7 26.7	*A. butzleri* *A. cryaerophilus*	Fernandez *et al.* (2015)
	Belgium	Faecal samples	77	*A. butzleri* *A. cryaerophilus* *A. skirrowii* *A. thereius*	De Smet *et al.* (2011b)
Cattle	Ghana	Faecal samples	29	*C. jejuni* *C. coli* *C. lari*	Karikari *et al.* (2017)
	Scotland	Faecal samples	22	*C. jejuni* *C. coli*	Rotariu *et al.* (2009)
	UK	Caeca samples	55	*C. hyointestinalis* *C. coli* *C. jejuni*	Milnes *et al.* (2008)
	EU	Intestinal samples	69	*C. jejuni*	Thépault *et al.* (2018)
	Japan	Rectal samples	40	*C. jejuni* *C. coli*	Haruna *et al.* (2013)

Table 2.7 Prevalence of *Campylobacteraceae* species in pigs, cattle, sheep and goats (continued)

Animal species	Region	Sample type	Prevalence (%)	Species	Reference
Cattle	Ghana	Faecal samples	13	*C. jejuni* *C. coli* *C. lari*	Karikari *et al.* (2017)
	Iran	Faecal samples	8 – 12	*A. butzleri* *A. cryaerophilus* *A. skirrowii*	Shirzad Aski *et al.* (2016)
	India	Faecal samples	10	*Arcobacter* spp.	Mohan *et al.* (2014)
	England	Faecal samples	43	*A. butzleri*	Merga *et al.* (2013)
	Italy	Faecal samples	22.6	*A. butzleri* *A. cryaerophilus* *A. skirrowii*	Giacometti *et al.* 2015
	Southern Chile	Faecal samples	6.7 – 9.6	*A. cryaerophilus*	Fernandez *et al.* (2015)
Sheep	Australia	Faecal samples	0-4	*C. jejuni* *C. coli*	Bailey *et al.* (2003)
	Scotland	Faecal samples	25	*C. jejuni* *C. coli*	Rotariu *et al.* (2009)
	Scotland	Faecal samples	14-21	*C. jejuni* *C. coli*	Sproston *et al.* (2011)
	Great Britain	Intestinal samples	44	*C. hyointestinalis* *C. coli* *C. jejuni*	Milnes *et al.* (2008)
	Ghana	Faecal samples	19	*C. jejuni* *C. coli* *C. lari*	
	New Zealand	Faecal samples	30	*Campylobacter* spp.	Moriarty *et al.* (2011)
	Iran	Faecal samples	10.1-18.5	*A. butzleri* *A. cryaerophilus* *A. skirrowii*	Shirzad Aski *et al.* (2016)
	Ghana	Faecal samples	19	*C. jejuni* *C. coli*	Karikari *et al.* (2017)

2.4.4 Factors that influence colonisation

Previous research has identified that the age of animal, season and biosecurity measures, as some of the factors that can influence the extent of flock/herd colonisation with *Campylobacter* and *Arcobacter* species. For poultry, the initial colonisation of *Campylobacter* spp. generally only occurs after 2–3 weeks of age (Workman *et al*., 2005). Before 14 days, inhibition has been reported and attributed to a lag phase. The lag phase is due to maternal antibodies passed down to hatchlings by the breeder, providing a protective immunity; this protective immunity tends to decrease as age increases (Sahin *et al*., 2003). The colonisation of poultry by *Arcobacter* spp. is not extensively researched however, evidence presented by Wesley & Baetz (1996) showed that younger chicks (8–16 weeks old) had a lower prevalence of *Arcobacter* spp. when compared to older chickens (56 weeks old). However, it should be noted that this longitudinal study did not sample the same flock and did not sample farms with the same level of biosecurity; factors that can interfere with colonisation and interpretation of results.

Calves and lambs exhibit higher *Campylobacter* spp. carriage levels when compared to adult sheep and cattle. For cattle of 1–2 and 7 months old, carriage levels of 8 log cfu/g (faecal matter) and 2–3 log cfu/g have been reported, respectively (Stanley *et al*., 1998). Furthermore, Thépault *et al*. (2018) reported a *Campylobacter* prevalence of 99% and 39% for calves and adult cattle, respectively. The prominence in the young could be attributed to the underdeveloped gastrointestinal tract of young ruminants leading to easy colonisation of *Campylobacter* spp. and also an acquired immunity by adult cattle (Stanley & Jones, 2003; Johnsen *et al*., 2006). For *Arcobacter* spp., similar results have been reported, as Giacometti *et al*. (2015) reported a high proportion of positive samples in calves when compared to adult cattle. The significant incidence of *Arcobacter* spp. was attributed to frequent exposure to environmental sources of *Arcobacter* spp. rather than an ease of colonisation due to an undeveloped gastrointestinal tract. However, the latter could also be feasible, but requires further research.

Season has been implicated in influencing the colonisation of chicken flocks, as flock colonisation seems to be higher during the warmer months of the year. Even though this phenomenon has not been fully explored, it does seem that species that belong to the *Campylobacteraceae* family thrive in warmer environments. A peak in *Campylobacter* spp. colonisation was reported by Weber *et al*. (2014) when broiler chickens and ducks (52 flocks) were sampled over 2001 to 2007. Weber *et al*. (2014) showed a peak in *Campylobacter* spp. colonisation during summer months, but also showed a reduced number of colonised flocks during winter months. Furthermore, a study aimed to correlate weather to *Campylobacter* spp. prevalence in Sri Lankan broilers, indicated that an increase of 1 °C in environmental temperature could result in 16% increase in *Campylobacter* spp. positive flocks (Kalupahana *et al*., 2018). Similar to poultry, Stanley *et al*. (1998) reported that the *Campylobacter* carriage rate in bovine hosts was influenced by season, whereby peaks were seen during the spring and autumn months. Interestingly, for this study through regression analysis, causation could not be linked to seasonal factors such as higher/lower temperatures, rainfall and sunshine. For this study, Stanley *et al*. (1998), speculated that peaks in *Campylobacter* carriage could be linked to indirect temperature dependant factors such as insects, migratory birds, rodents etc. Similar to *Campylobacter* spp., *Arcobacter* spp. also tends to be more prevalent during the warmer parts of the year; ÇelIk *et al*. (2018)

attributed maximum isolation rates of 19.26–34.31% in geese to sampling during fall and summer (warmer parts of the year).

At a farm, biosecurity can be a set of practices implemented to reduce risk of transmission of infectious diseases from reservoirs/vehicles to farmed animal. Several practices have been highlighted in previous studies. These practices can include the use of site-dedicated clothing and footwear for specific areas of the farm. For instance, at a broiler farm, each broiler house could have dedicated clothing which can be issued to personnel before entry. This practice has shown to have a positive effect in decreasing the transfer of infections at farm level (Bouwknegt *et al*., 2004). For poultry and pig farms, the placement of boot dips at each house/building entrance can inhibit the transfer of infections. However, Evans & Sayers (2000) showed that this practice could increase the probability of an infection, when disinfectant used in boot dips is not replenished routinely. Furthermore, some poultry farms make use of hygiene barriers. Hygiene barriers separate the outside environment from the inside 'clean and protected' environment. Simply, hygiene barriers could be a line at which personnel are required to change clothing and wash footwear (Newell *et al*., 2011). Furthermore, where possible flynets can be placed at each ventilation point (poultry houses) and rodent control programmes can be implemented for each farm (Federighi, 2017; Sibanda *et al*., 2004).

Farms with strict and consistent biosecurity measures tend to reduce the risk of *Campylobacter* spp. infection in farmed animals. Perko-Mäkelä *et al*. (2009) reported a low prevalence of duck flocks colonised with *Campylobacter* spp. when kept under strict biosecurity. The implementation of biosecurity measures can curb the chances of *Campylobacter* spp. colonisation as suggested by Gwimi *et al*. (2015) for pigs from Nigeria, Colles *et al*. (2008a) for free range broiler chickens, Ramonaitė *et al*. (2013) for cattle herds in Lithuania and Seliwiorstow *et al*. (2015) for broiler chickens from Belgium. However, even though the idea of biosecurity at primary production is advised, it is in fact a difficult concept due to the lack of knowledge in effective interventions against *Campylobacter* spp. at farm level. Additionally, a "one-farm-fits-all" strategy does not exist (Wagenaar *et al*., 2006).

With regards to the colonisation of *Arcobacter* spp. there is a lack of research that investigates the influence of biosecurity measures on the colonisation of flocks/herds, nonetheless a study by Wesley & Baetz (1996) could not prove a definitive effect of biosecurity measures as a 53% prevalence was proven in a grandparent flock (56 weeks) placed under high biosecurity measures, and a 1–3% prevalence was determined for a flock (8 and 16 weeks) under low biosecurity measures. For this case, it could be possible that other factors such as age and the state of the housing facilities influenced prevalence levels, as when another grandparent flock (56 weeks), kept under high biosecurity measures and in modern housing facilities was sampled, *Arcobacter* spp. could not be detected.

2.4.5 *Campylobacter* spp. in other animals

Pet animals, especially cats and dogs have also been identified as possible vehicles for species belonging to the *Campylobacteraceae* family. A Barbados based study determined the prevalence of *C. jejuni*, *C. coli*, *C. upsaliensis* and *C. helveticus* in dogs to be 51.5%, 7.6%, 4.5% and 50%, respectively (Workman *et al.*, 2005). Workman *et al.* (2005) also found *C. jejuni*, *C. upsaliensis* and *C. helveticus* in cats at incidence levels of 10%,

30% and 50%, respectively. Workman *et al.* (2005) showed that dogs could be significant reservoirs of *Campylobacter* spp. and can contribute to human enteric infections, as a correlation between human *Campylobacter* isolates and isolates from dogs was proven, where *Campylobacter* isolates from household dogs were more genetically related to human isolates. Lazou *et al.* (2017), also proved the prevalence of *Campylobacter* spp. in cats (n = 132) and dogs (181), however for this study low prevalence levels of 3.8% for cats and 12.1% for dogs were reported. Similarly, Andrzejewska *et al.* (2013) also reported low prevalence levels of 4.8% and 9.86% in dogs and cats, respectively. Andrzejewska *et al.* (2013) attributed the low isolation rate to sampling occurring during the winter and autumn months of the year. Overall, prevalence differences between studies have been recognised and have been attributed to varying age of animals, season, animal species and even methods used for sampling (Andrzejewska *et al.*, 2013). However, there is sufficient evidence that recognises pet animals as a potential cause of *Campylobacter* spp. and even *Arcobacter* spp. infections in humans. Just like *Campylobacter* spp., *Arcobacter* spp. has been isolated just as frequently in cats and dogs. By sampling the oral cavity Pejchalova *et al.* (2016) proved a prevalence of 60% and 39% for dogs and cats, respectively. Similar results have been reported by other researchers (Petersen *et al.*, 2007; Fera *et al.*, 2009). Previous research has also shown that cats and dogs harbour the species mostly associated with human infections, as *A. butzleri*, *A. skirrowii* and *A. cryaerophilus* have been isolated from pet animals. The most likely route of transmission would be contact with the oral cavity i.e. licking and biting (Petersen *et al.*, 2007).

2.5 *Campylobacter* and *Arcobacter* species along the slaughter process

2.5.1 Slaughter steps

2.5.2 Scalding

During the slaughter process, an area of hygienic concern is the scalding process. The scalding process is the treatment of poultry and pig carcasses with either hot water or steam in order to loosen hair/feathers from follicles. Poultry carcasses can be scalded by manually immersing carcasses in warm water, however in large abattoirs hanging poultry carcasses are dipped in a single or multistage scalding bath or pass through spray showers, whereby carcasses are subjected to hot water at a pressure of 137.9 kPa. The time and temperature combination can vary and is dependent on the feathers to be removed and also the defeathering equipment being used. However, typically a temperature range of 50–51°C for 3.5 min is used or 56–58°C for 2 to 2.5 min can also be used for poultry carcasses (Arun & Irshad, 2013).

The use of high temperatures can lead to the expectation of the ability to decrease microbial contamination on carcasses, in fact for *Campylobacter* spp. the scalding process has been reported to reduce *Campylobacter* levels on poultry. For instance, when poultry carcasses were immersed in a counter flow current with a temperature of 55.4°C for 3 min, scalding reduced the prevalence of positive carcasses (Berrang & Dickens, 2000). However, for *Arcobacter* spp. there is evidence that suggests that the scalding step can aid in the contamination of poultry carcasses, as Ho *et al.* (2008) found that scalding water from scalding tanks can be contaminated with *Arcobacter* spp. after multiple carcasses were scalded in the same water. Additionally, Ho *et al.* (2008) reported that *Arcobacter* spp. could survive scalding water conditions, in this

case scalding was done at a temperature of 52°C for 3 min. Similarly, in the study of *Campylobacter* spp., evidence of cross contamination during scalding has been reported in poultry carcasses. Osiriphun *et al*. (2011) reported a *C. jejuni* prevalence level of a 100% for poultry carcasses sampled immediately after scalding, using a counter current scalding chamber. For this study the post-scalding phase had the highest prevalence of *C. jejuni* when compared to other slaughter steps.

Contamination during scalding can be attributed to the submerging of dirty poultry carcasses into a common water source and *Campylobacter*'s ability to survive in scalding water. These observations were reported by Bily *et al*. (2010) who found that scalding water samples were negative for *Campylobacter* spp. before scalding and found *Campylobacter* spp. after the submersion of 1000 turkey carcasses. It should be noted that pre-scalded carcasses can be dirtied with soil and faecal matter. External contamination with faecal matter is not uncommon as Whyte *et al*. (2001) reported that transportation of poultry can lead to stress which can induce faecal excretion, resulting in faecal matter being present on feathers. Furthermore, during scalding, contamination is aided by hair follicles opening up during scalding and only closing during the chilling phase of the slaughter process, allowing *Campylobacter* spp. to migrate into the opened hair follicles and to be retained in the hair follicles during chilling (Osiriphun *et al*., 2011).

Traditionally, pig carcasses are subjected to horizontal scalding, which requires the immersion of carcasses in a bath of water. However, with technological advances and the need to produce carcasses with high bacteriological standards, vertical water scalding, and vertical condensation scalding is preferred. Where in the former, circulating water is sprayed on hanging carcasses and for the latter, steam stemming from a double walled tunnel is generated and blown onto hanging carcasses. The time and temperature combination can vary and is dependent on the hair to be removed and also the dehairing equipment being used. Typically, a temperature range of 60–70°C for 5 to 10 min is used (Arun & Irshad, 2013).

For pig carcasses, the scalding process has been reported to be successful in the reduction of *Campylobacter* levels. For instance, Pearce *et al*. (2003) reported a reduction in the prevalence of *Campylobacter* spp. during the slaughter of pig carcasses. The reduction in *Campylobacter* levels was attributed to the thermal treatment of carcasses during the submerged scalding step reducing *Campylobacter* levels. However, the reduction of *Campylobacter* levels can only be partially attributed to the scalding step, as Pearce *et al*. (2003) collected samples post- polishing, which is preceded by singeing. Singeing is also a thermal treatment (1200°C for 10 s) that can possibly reduce bacterial contamination on carcass surfaces. The lack of reports of cross contamination or survival of *Campylobacter* spp. during the scalding step (scald tank/scald water) for pigs, can be attributed to the higher temperatures of 60–62°C which could potentially help to inhibit enteric pathogens such as *Campylobacter* spp. (Bolton *et al*., 2002).

2.5.3 Defeathering and dehairing

During the slaughter of poultry, the defeathering process makes use of finger-like equipment which exert pressure to effectively remove wet feathers post-scalding. Poultry carcasses with low/undetectable *Campylobacter* levels can be found to be positive for *Campylobacter* spp. after defeathering (Keener *et al*.,

2004). For instance, Berrang & Dickens (2000) reported an increase in *Campylobacter* spp. prevalence after defeathering. The contamination during this step was attributed to the pressure exerted by the finger-like equipment. The pressure can result in the excretion of faecal matter from the cloaca, subsequently contaminating carcasses and neighbouring carcasses. Additionally, the finger-like equipment can act as a vehicle for cross-contamination; transferring *Campylobacter* spp. to each passing carcass (Berrang & Dickens, 2000). Cross contamination during the defeathering step was also noted by Semaan *et al*. (2014), who isolated *C. lari* from broiler chickens after the defeathering step, Semaan *et al*. (2014) expressed that *C. lari* was not previously isolated at reception (process step before defeathering).

The complete dehairing of pig carcasses requires multiple steps after scalding, such as scraping of scalded carcasses, singeing of scraped carcasses and polishing of singed carcasses (Pearce *et al*., 2003). Dehairing can result in a reduction of *Campylobacter* spp. levels on pig carcasses as seen by Pearce *et al*. (2003), when a reduced level of *Campylobacter* spp. was reported for pig carcasses post-polishing. For this study, the reduction of *Campylobacter* spp. levels was mostly attributed to high temperatures (which can be as high as 1200°C) used during singeing (Pearce *et al*., 2003).

The contamination of pig carcasses during the dehairing step has been associated with the dehairing machine. Pearce *et al*. (2003) reported that trapped debris on the frame of dehairing equipment can be positive for *Campylobacter* spp. and can contaminate pig carcasses. However, cross contamination is not limited to just the trapped debris, as faecal matter can also escape during dehairing and subsequently contaminate the dehairing equipment and also the circulating water in the dehairing equipment.

The implication of defeathering and/or dehairing in *Campylobacter* spp. contamination is much more documented and reported than *Arcobacter* spp.'s ability to contaminate during this step, nonetheless a study by Lipman *et al*. (2008) reported the presence of *Arcobacter* spp. in water dripping off poultry carcasses after defeathering. Just like *Campylobacter* spp., it could be speculated that the *Arcobacter* spp. was from faecal matter escaping during defeathering. However, it should be noted that Lipman *et al*. (2008) sampled water drippings from carcasses, thus one can't conclusively determine if the *Arcobacter* spp. was from the water or the carcass. Past research has attributed *Arcobacter* spp. contamination to water used at abattoirs rather than faecal contamination, especially when *Arcobacter* spp. could not be isolated from cloaca and/ or faecal samples but could be isolated from the slaughter environment. For instance, Van Driessche & Houf (2007a) found no *Arcobacter* spp. on poultry carcasses and urged the study of alternative contamination sources such as water.

2.5.4 Hide/skin removal

Animal skins have been implicated in harbouring many enteric pathogens including *Campylobacter* spp. (Wieczorek *et al*., 2009). Therefore, the act of removing the skin (skinning) could result in carcasses being contaminated with *Campylobacter* and *Arcobacter* species. For instance, the presence of *Arcobacter* spp. in retail beef mince and beef carcasses at isolation levels of 9% and 37%, respectively, resulted in De Smet *et al*. (2010) attributing *Arcobacter* spp. levels found on the carcass to dehiding, as no visible damage to the intestine

was seen and it was hypothesized that knives allowed for cross contamination as the incisions made to the skin were made from the outer to the inner surface of the hide, during manual dehiding.

In the case of *Campylobacter* spp., Wieczorek *et al*. (2009) reported *C. jejuni* as the most frequently isolated pathogen on bovine hides when compared to other major food pathogens such as *Escherichia coli*, *Salmonella* and *Listeria monocytogenes*. Evidence of cross contamination during the skinning phase was also reported by Wieczorek *et al*. (2009), who proved the presence of *C. jejuni* on bovine hides (24.6%) resulted in the contamination of carcass surfaces, as 2.9% of carcasses were contaminated with *Campylobacter* spp. after skinning. During skinning, faecal matter can be transferred from the hide to carcass surfaces, which can result in the presence of enteric pathogens, such as *Campylobacter* spp. on carcass surfaces. Other modes of transferring enteric pathogens during skinning includes personnel (hands) coming into direct contact with the outer surface of the skin/hide and then the flesh of the carcass. Furthermore, just like for *Arcobacter* spp., the skinning step can promote other vehicles of contamination, such as knives which can help in spreading *Campylobacter* spp. from carcass to carcass if improper hygienic practices are followed (De Smet *et al*., 2010).

2.5.5 Evisceration and bunging

During the removal of the viscera, *Campylobacter* and *Arcobacter* species can be transferred onto the carcass and the slaughter environment, through the spillage of intestinal fluid (Perko-Mäkelä *et al*., 2009). The rupture of viscera is not uncommon during slaughter (Rosenquist *et al*., 2006), and it can lead to the contamination of carcasses with *Campylobacter* and *Arcobacter* species. Khoshbakht *et al*. (2014) found that most poultry carcasses were contaminated with *Arcobacter* spp. after evisceration, as 73% of the carcasses were contaminated with *Arcobacter* spp. whilst lower contamination levels were reported for pre-scalding (30%), post-scalding (48%) and post-chilling (18%). Furthermore, Lipman *et al*. (2008) also showed that *Arcobacter* spp. on poultry carcasses stems from the intestinal tract as the prevalence of *Arcobacter* spp. in the gastrointestinal tract was proven and genotypic similarities between isolates from the carcass and the gastrointestinal tract were determined; cementing the notion that *Arcobacter* spp. contamination was introduced through faecal contamination.

For *Campylobacter* spp. contamination, Figueroa *et al*. (2009) showed that contaminated poultry carcasses can significantly increase after evisceration, as 90% of carcasses sampled were positive for *Campylobacter* spp. after evisceration and out of the four processing steps (reception, after defeathering, after evisceration and after chilling), evisceration resulted in the highest number of contaminated poultry carcasses, with an average carriage level of 5.2 log cfu per carcass. The influence of the evisceration step was also noted by Reich *et al*. (2008) during the investigation of critical processing steps in the control of *Campylobacter* spp. in broilers. Reich *et al*. (2008) also reported a high contamination level of 87.3% after evisceration. Several other studies have determined points of contamination by correlating genotypes of *Campylobacter* isolates found during slaughter. Lindmark *et al*. (2006) and Rasschaert *et al*. (2006) have proven a close correlation between isolates from cloaca, small intestines and poultry carcasses. The evisceration step can also increase the *Campylobacter* load on the carcasses, as seen by Seliwiorstow *et al*.

(2015) who reported an increase of >1 log after the evisceration of broiler chickens. In nearly all large poultry processing plants, evisceration is no longer done manually and casual observation in abattoirs indicate that there tends to be a incidence of intestinal rupture during the automatic removal of the gastrointestinal tract by the spoons whilst microbial contamination is further enhanced by the (ruptured) viscera being left to hang outside against the carcass. Furthermore, during evisceration the slaughter environment can be contaminated, as Perko-Mäkelä *et al*. (2009) reported that 50 to 100% boots worn by personnel in the evisceration area were contaminated with *Campylobacter*, demonstrating that once contamination occurs it cannot be contained.

The contamination of carcasses with *Campylobacter* and *Arcobacter* species can also occur during the bunging and evisceration of cattle, sheep, goat and pigs. In the case of bunging and evisceration, *C. jejuni* and *C. coli* have been recognised as pathogens that reside in the gastrointestinal tract and subsequently in faecal matter, therefore bunging and evisceration can result in the contamination of carcasses with *Campylobacter* spp. (Luque *et al*., 2018). Additionally, if these process steps are not conducted in the correct manner, contamination of utensils and slaughter environments can occur. The importance of evisceration in relation to *Campylobacter* spp. contamination was reported by Maramski (2012) as *C. coli* and *C. jejuni* were isolated from pig carcasses after evisceration. Maramski (2012) also proved cross contamination, as surfaces and knives used during the evisceration step were found to be positive for *Campylobacter* spp. Interestingly, this study showed that strict hygiene practices allowed for containment of *Campylobacter* contamination to one phase of the slaughter process, as contamination was not seen during carcass halving and head cracking (steps that occur after evisceration in this case).

The evisceration step has also been implicated in the contamination of beef carcasses with *Arcobacter* spp. At post-evisceration *Arcobacter* spp. was quantified at 3 $\log_{10}$ cfu/cm^2, with the chest and foreleg being frequently contaminated (De Smet *et al*., 2010). Similarly, Van Driessche & Houf (2007b), reported the highest contamination level (91%) in pork carcasses directly after evisceration and once again, the chest and the foreleg were frequently found to be contaminated sites of the carcasses, at a quantifiable *Arcobacter* spp. count of 2 $\log_{10}$ cfu per 100 cm^2 and 300 cm^2. Both De Smet *et al*. (2010) and Van Driessche & Houf (2007b) attributed the contamination to the possible perforation of gastrointestinal tract resulting in intestinal fluid flowing downwards; contaminating chest and forelegs.

2.5.6 Washing and chilling

Abattoirs are well aware of the pathogenic threats that can arise from slaughter, which is why the slaughter process includes steps such as washing and chilling of carcasses. Washing and chilling steps are also seen as the critical control points during slaughter (Bolton *et al*., 2002). The effect of these process steps is indeed positive as many researchers have proven the reduction of *Campylobacter* prevalence and *Campylobacter* load on carcasses during these steps. For instance, the chilling of calves and cattle carcasses that were contaminated with *C. jejuni* and *C. hyointestinalis* (at a load of 1.3 to 3.2 log cfu/cm^2) resulted in a load reduction of 50 to 100 fold (Grau, 1988). Even though the conditions of chilling were not given by Grau (1988), it is clear that chilling can help reduce *Campylobacter* levels on carcasses. The positive effect of chilling was also reported by Maramski (2012), as during the slaughter of pigs, seven pig carcasses were contaminated with

Campylobacter and after chilling only one carcass was found to harbour *Campylobacter* spp. Similar trends have been found for *Arcobacter* spp. as De Smet *et al.* (2010) has reported a reduction in *Arcobacter* spp. contaminated bovine carcasses after forced air cooling. Additionally, Van Driessche & Houf (2007b) reported a reduction due to a combination of cooling and drying of pig carcass surfaces. The ability to reduce *Campylobacter* spp. and *Arcobacter* spp. contamination by chilling could be attributed to the chemical changes in the lipid bilayer of microbial cells induced by low temperatures, which results in a permanent physical damage of cells (De Smet *et al.*, 2010).

For poultry, Khoshbakht *et al.* (2014) showed that with an increasing isolation rate of *Arcobacter* spp. from four slaughter points namely, pre-scalding (30%), post-scalding (48%), post-evisceration (73%) and post-chilling (18%); chilling was the only step to reduce the number of positive poultry carcasses. Similarly, for the contamination of poultry carcasses with *Campylobacter* spp., Figueroa *et al.* (2009) showed a *Campylobacter* spp. reduction of 1.6 and 2 logs after chilling (water chillers with 0.5–0.75 ppm of free chlorine) in two slaughterhouses from Chile. Additionally, for the same broiler carcasses Figueroa *et al.* (2009) showed the importance of washing and the use of chlorinated water as it resulted in a significant 2 log reduction in carcasses contaminated with *C. jejuni*.

Even though washing and chilling can reduce contamination levels, complete eradication of *Campylobacter* spp. and *Arcobacter* spp. contamination by these steps is not necessarily possible (Rosenquist *et al.*, 2006). Figueroa *et al.* (2009) expressed that chilling (chlorine chillers) can fail in reducing *Campylobacter* populations, especially when the *Campylobacter* load is high. Other studies have also shown that chilling and washing can have no effect on the *Campylobacter* and *Arcobacter* contamination on poultry (Son *et al.*, 2007; Semaan *et al.*, 2014; Seliwiorstow *et al.*, 2015). Lastly, in complete contradiction, the chilling process can also cause an increase in contamination of poultry carcasses with species that belong to the *Campylobacteraceae* family. However, when an increase occurs, it is usually attributed to the cross contamination between contaminated and uncontaminated carcasses (Padungtod & Kaneene, 2005; Khoshbakht *et al.*, 2014).

2.5.7 Trimming and deboning

Trimming and deboning processes can contribute to the cross contamination of uncontaminated carcasses with *Campylobacter* spp. (FSANZ, 2013). The deboning process has been implicated in the bacterial contamination of the surface of equipment and the slaughter environment (Gouws *et al.*, 2017), subsequently producing vehicles for bacterial contamination, as seen by Gill & Harris (1982), who reported the presence of *C. fetus* and *C. jejuni* on equipment and deboning tables during the processing of contaminated calf carcasses. Gill & Harris (1982) reported an environmental and carcass contamination during deboning and quantified it at ≤ 1 log cfu/cm^2, at such low levels and low environmental temperatures (during slaughter) it is unlikely that *Campylobacter* spp. would proliferate. However, low levels of *Campylobacter* spp. can possibly still result in an infection in humans as *Campylobacter* spp. has been proven to have a low infective dose (Black *et al.*, 1988).

31

2.5.8 Cleaning and disinfection

Once contamination begins in the slaughterhouse, it is clear that it cannot be contained as the slaughtering of contaminated animals can result in the presence of *Campylobacter* spp. on equipment, knives, surfaces and personnel (Thépault *et al*., 2018). Luber *et al*. (2006) reported that equipment such as knives and cutting boards can be vectors of *Campylobacter* spp. contamination. The study by Balogu *et al*. (2014) at two poultry abattoirs affirmed the occurrence of cross contamination of the environment during the slaughter process, as *C. jejuni* was isolated from packaging tables, dressing tables, floor surfaces and washing tubs. All meat contact surfaces and equipment showed high prevalence of *C. jejuni* during the slaughter process (Balogu *et al*. 2014). Even though the spread of *Arcobacter* spp. in the slaughter environment has not been extensively researched, it does seem that *Arcobacter* spp. can also contaminate surfaces and equipment. For instance a study by Elmali & Can (2016) showed isolation of *Arcobacter* spp. from 40% of slaughter surfaces and knives.

Hygiene practices such as cleaning and disinfecting are employed within the slaughter process in order to curb contamination, however, certain *Arcobacter* species can survive environmental stresses as *Arcobacter* spp. can form biofilms which help in the resistance of chemicals used for cleaning and disinfecting (Córdoba-Calderón *et al*., 2017). Also, *C. jejuni* genotypes can successfully survive on cleaned and disinfected surfaces overnight (Peyrat *et al*., 2008b). This is attributed to the fact that *C. jejuni*, is one of the most resilient of the *Campylobacter* species, as it can survive environmental stresses, cleaning and disinfection. The ability to survive stressful slaughter environments has been attributed to *C. jejuni*'s genome plasticity, which allows for adaptation when stress occurs and it is suspected that *C. jejuni* survives on surfaces after cleaning through the formation of biofilms (Peyrat *et al*., 2008a). The ability to resist cleaning and disinfecting procedures was also seen by Balogu *et al*. (2014), as 70% of floor samples taken during the slaughter of poultry were still positive for *C. jejuni*, even though recommended sanitisers were used to clean floors. It should be noted that the poor efficacy of disinfectants could also be attributed to the active compounds in sanitisers and disinfectants, as Peyrat *et al*. (2008b) disclosed that some genotypes are capable of surviving disinfectants with quaternary ammonium compounds. Interestingly, disinfectants with quaternary ammonium compounds are widely used and have been generally reported to be effective agents in the reduction of *Campylobacter* spp. (Avrain *et al*., 2003). Maramski (2012) also proved the importance of cleaning and disinfection, as cleaning and disinfection procedures yielded a positive effect by completely eradicating *Campylobacter* spp. from surfaces and equipment. In this case, the positive effect was attributed to *Campylobacter*'s sensitivity to the environment and a strict adherence to the hygiene practices in place.

2.6 Regulatory information

2.6.1 *Campylobacter* spp.

2.6.2 Slaughter line

The Commission regulation (EC) No. 2073/2005 provides the microbiological criteria and set of rules in respect to process hygiene and contamination values for food business operations. Regulation (EC) No. 2073/2005 has recently been amended to regulation (EC) No. 2017/1495. Regulation (EC) No. 2017/1495 now

includes criteria for *Campylobacter* spp. during the slaughter of broiler carcasses. The revision of regulation (EC) No. 2073/2005 was based on EFSA and ECDC's risk assessment, which determined campylobacteriosis as the most reported human food-borne illness in 2015. The guidelines included in regulation (EC) No. 2017/1495 hope to reduce the risk of campylobacteriosis by 50% if poultry abattoirs can successfully comply with the microbial specification of 1000 cfu/g. In 2011 EFSA also suggested that reduction in campylobacteriosis cases can range from 50-90% if fresh broiler meat comply with the specification of 1000 cfu/g for at least 8 years (2018 to 2025). It should be noted that currently guidelines for only broiler chickens exists (Anonymous, 2017).

2.6.3 Indicator microorganisms and *Campylobacter* spp.

Several researchers explored the relationship between indicator microorganisms and *Campylobacter* spp. For instance, during the investigation of the use of hygiene indicator microorganisms for selected pathogens (*Salmonella* spp. and *Campylobacter* spp.) on beef, pork and poultry carcasses, Ghafir *et al.* (2008) reported that generic *E. coli* was a good index for *Campylobacter* contamination as the microorganism also originates from the intestinal tract of food-producing animals. Thus *E. coli* could be effective in indicating faecal contamination and providing evidence of efficacy of the slaughter process. Duffy *et al.* (2014) also reported a strong correlation (r = 0.8) between *E. coli* and *Campylobacter* during the slaughter of Australian broilers. Ultimately, high *E. coli* counts on carcasses can possibly indicate for the presence of *Campylobacter* spp. on carcasses. However, other previous studies have also reported opposing findings, such as Pacholewicz *et al.* (2016), who found no correlation between *E. coli* and *Campylobacter* on broiler carcasses (after chilling), but did see that *E. coli* and *Campylobacter* spp., reacted the same way to the slaughter steps. The most recent study on this issue was conducted by Roccato *et al*. (2018), who found that *E. coli* quantification may be a useful tool in identifying carcasses contaminated with *Campylobacter* spp. as high levels of *E. coli* (> 3 log cfu/g) indicate a high probability of *Campylobacter* spp. on carcasses. The idea of *E. coli* as an indicator microorganism for the presence of *Campylobacter* spp. may remain attractive as enumeration techniques for generic *E. coli* are easier and also cost less (Habib *et al.*, 2012).

2.6.4 Ready to eat (RTE) products

Ready-to-eat (RTE) foods are consumed without further treatment such as cooking. There is an affinity towards RTE food products due to the good taste and simple preparation required in order to consume. It is common that such products can contain meat, which raises concerns related to microbiological contamination (Abay *et al.*, 2017). However, regardless of the ingredients, the expectation is that RTE products should be free from pathogens such as *Campylobacter* and *Arcobacter* species, as reported by Abay *et al.* (2017) and Whyte *et al.* (2004). Microbiological risk could arise if inadequate hygiene practices, heating/thermal treatment and storage conditions takes place. RTE products could then be important vehicles in the transmission of *Campylobacter* spp. as reported by Mpalang *et al.* (2014). Therefore, microbial specifications for *Campylobacter* spp. in RTE foods have been set as absent in 25 g of a food product (Sagoo *et al.*, 2007).

2.6.5 *Arcobacter* spp.

Unlike *Campylobacter* spp. there are currently no regulations in place for *Arcobacter* spp. contamination during the slaughter process, finished products and RTE products. However, there should be an urgency to correct the lack of regulatory information for *Arcobacter* spp., as past research has proved that contamination of retail products can occur and can be aided by the slaughter process. Furthermore, from research that compares the genome of *Arcobacter* spp. to *Campylobacter* spp. it is clear that these species might share the same virulence factors (Barboza *et al.*, 2017), possibly indicating that the infective dose of *Arcobacter* spp. could also be low and microbial specifications would have to be as strict as absent in 25g, in food products. However, this notion is circumstantial as research investigating the presence of *Arcobacter* spp. in food is still scarce.

2.7 Conclusion and future research

The increase in meat consumption warrants the need to provide meat and meat products that are safe for human consumption. The safety of meat can be threatened by the presence of pathogenic bacteria such as *Campylobacter* spp. and *Arcobacter* spp. (Dekker *et al.*, 2019). If food contaminated with *Campylobacter* spp. and *Arcobacter* spp. is consumed, it can possibly result in gastroenteritis and severe diseases such as GBS, as well as death in extreme cases. Therefore, future research should explore prevention and reducing bacterial contamination at primary production and at slaughter.

Food-producing animals (such as poultry, cattle, pig, and sheep) can harbour *Campylobacter* and *Arcobacter* species, and can act as transmission reservoirs for human infections. At primary production, adhering to strict biosecurity measures should inhibit horizontal transmission. However, due to the various routes of transmission at primary production, future research should focus on partnering biosecurity measures with novel interventions such as the use of vaccines and feed additives (such as probiotics, prebiotics and bacteriocin); that can ultimately disturb the establishment of these species in the gastrointestinal tract of a host, this can possibly prevent and reduce the presence of *Campylobacter* and *Arcobacter* species on the final product (Vandeplas *et al.*, 2008).

Final/finished/retail products can be contaminated if slaughter steps such as skinning, defeathering and evisceration are not conducted in a hygienic manner. At this stage, research should focus on the use of decontamination techniques to help reduce bacterial contamination that can occur. Research has indicated that chemical interventions (such as acetic acid, citric acid and lactic acid) can reduce *Campylobacter* and *Arcobacter* contamination (Červenka *et al.*, 2004; CoşAnsu & Ayhan, 2010). Therefore, future research should explore the practical use of chemical interventions during carcass washes and sprays before and/or after points of concern, such as evisceration. Furthermore, alternative interventions can include the use of irradiation as a tool to reduce bacterial contamination on carcasses (EFSA, 2011). Additionally, there is evidence that *C. jejuni* can be susceptible to zinc oxide, silver and copper oxide nanoparticles (Duffy *et al.*, 2018). More research aimed at exploring the use of nanoparticles as disinfectants and even surface biocides (King *et al.*, 2018) should be conducted.

2.8 References

Abay, S., Irkin, R., Aydin, F., Müştak, H.K. & Diker, K.S. (2017). The prevalence of major foodborne pathogens in ready-to-eat chicken meat samples sold in retail markets in Turkey and the molecular characterization of the recovered isolates. *LWT - Food Science and Technology*, **81**, 202–20.

Abdelbaqi, K., Buissonnière, A., Prouzet-Mauleon, V., Gresser, J., Wesley, I., Mégraud, F. & Ménard, A. (2007). Development of a real-time fluorescence resonance energy transfer PCR to detect *Arcobacter* species. *Journal of Clinical Microbiology*, **45**, 3015–3021.

Abid, M., Wimalarathna, H., Mills, J., Saldana, L., Pang, W., Richardson, J.F., Maiden, M.C.J. & McCarthy, N.D. (2013). Duck liver-associated outbreak of *Campylobacter* infection among humans, United Kingdom, 2011. *Emerging Infectious Diseases*, **19**, 1310–1313.

Aboderin, A.O. Smith, S.I., Oyelese A.O., Onipede, A.O., Zailani S.B. & Coker, A.O. (2002). Role of *Campylobacter*, Nigeria. *East African Medical Journal,* **79**, 423–426.

Alagić, D., Smajlović, A., Smajlović, M., Maksimović, Z., Članjak, E., Čaklovica, K., Tanković, S., Veljović, E., Ljevaković-Musladin, I. & Rifatbegović, M. (2016). Prevalence of *Campylobacter* contamination in broiler meat. *Meso*, **18**, 357–361.

Allain, V., Chemaly, M., Laisney, M.J., Rouxel, S., Quesne, S. & Le Bouquin, S. (2014). Prevalence of and risk factors for *Campylobacter* colonisation in broiler flocks at the end of the rearing period in France. *British Poultry Science*, **55**, 452–459.

Allos, B.M. & Lastovica, A.J. (2011). *Campylobacter* Infections. In: Tropical Infectious Diseases: Principles, Pathogens and Practise (edited by R. L. Guerrant, D.H. Walker, & P.F. Weller). Pp. 145–149. Philadelphia: Saunders/Elsevier.

Altekruse, S.F. & Tollefson, L.K. (2003). Human campylobacteriosis: a challenge for the veterinary profession. *Journal of the American Veterinary Medical Association*, **223**, 445–452.

Andrzejewska, M., Szczepańska, B., Klawe, J.J., Śpica, D. & Chudzińska, M. (2013). Prevalence of *Campylobacter jejuni* and *Campylobacter coli* species in cats and dogs from Bydgoszcz (Poland) region. *Polish Journal of Veterinary Sciences*, **16**, 115–120.

Anonymous (2017). Commission regulation (EC) No. 2017/1495. [Internet document]. URL https://eurlex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:32005R2073&from=EN 01/03/2018.

Arun, A.I. & Irshad, T.S. (2013). Scalding and its significance in livestock slaughter and wholesome meat production. *International Journal of Livestock Research*, **3**, 45–53.

Atabay, H.I., Wainø, M. & Madsen, M. (2006). Detection and diversity of various *Arcobacter* species in Danish poultry. *International Journal of Food Microbiology*, **109**, 139–145.

Avrain, L., Allain, L., Vernozy-Rozand, C. & Kempf, I. (2003). Disinfectant susceptibility testing of avian and swine Campylobacter isolates by a filtration method. *Veterinary Microbiology*, **96**, 35–40.

Aydin, F., Atabay, H.I. & Akan, M. (2001). The isolation and characterization of *Campylobacter jejuni* subsp. *jejuni* from domestic geese (*Anser anser*). *Journal of Applied Microbiology*, **90**, 637–642.

Bailey, G.D., Vanselow, B.A., Hornitzky, M.A., Hum, S.I., Eamens, G.J., Gill, P.A., Walker, K.H. & Cronin, J.P. (2003). A study of the foodborne pathogens: *Campylobacter*, *Listeria* and *Yersinia*, in faeces from slaughter-age cattle and sheep in Australia. *Commun Dis Intell*, **27**, 249–257.

Balogu, T.V., Nwaugo, V.O. & Onyeagba, R.A. (2014). Persistence and Biofilm Assessment of *Campylobacter jejuni* in Poultry Abattoir. *Nigerian Food Journal*, **32**, 54–61.

Banting, G. & Figueras, S. M. J. (2017). Global Water Pathogen Project. [Internet document]. URL http://www.waterpathogens.org/book/*Arcobacter* 08/08/2018.

Barboza, K., Angulo, I., Zumbado, L., Redondo-solano, M. & Castro, E. (2017). Isolation and Identification of *Arcobacter* Species from Costa Rican Poultry Production and Retail Sources. *Journal of Food Protection*, **80**, 779–782.

Bartholomew, N., Brunton, C., Mitchell, P., Williamson, J. & Gilpin, B. (2014). A waterborne outbreak of campylobacteriosis in the south island of New Zealand due to a failure to implement a multi-barrier approach. *Journal of Water and Health*, **12**, 555–563.

Berrang, M.E. & Dickens, J.A. (2000). Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. *Journal of Applied Poultry Research*, **9**, 43–47.

Bily, L., Petton, J., Lalande, F., Rouxel, S., Denis, M., Chemaly, M., Salvat, G. & Fravalo, P. (2010). Quantitative and Qualitative Evaluation of *Campylobacter* spp. Contamination of Turkey Cecal Contents and Carcasses during and following the Slaughtering Process. *Journal of Food Protection*, **73**, 1212–1218.

Black, R.E., Levine, M.M., Lou, M., Hughes, T.P. & Martin, J. (1988). Experimental *Campylobacter jejuni* Infection in humans. *The Journal of Infectious Diseases*, **157**, 472–479.

Boer, R.F. De, Ott, A., Güren, P., Zanten, E. Van, Belkum, A. Van & Kooistra-Smid, A.M.D. (2013). Detection of Campylobacter species and Arcobacter butzleri in stool samples by use of real-time multiplex PCR. *Journal of Clinical Microbiology*, **51**, 253–259.

Bogantes, E.V., Fallas-Padilla, K.L., Rodríguez-Rodríguez, C.E., Jaramillo, H.F. & Echandi, M.L.A. (2015). Zoonotic Species of the genus *Arcobacter* in Poultry from different regions of Costa Rica. *Journal of Food Protection*, **78**, 808–811.

Bolton, D.J., Pearce, R.A., Sheridan, J.J., Blair, I.S., McDowell, D.A. & Harrington, D. (2002). Washing and chilling as critical control points in pork slaughter hazard analysis and critical control point (HACCP) systems. *Journal of Applied Microbiology*, **92**, 893–902.

Bouwknegt, M., Giessen, A.W. Van De, Dam-Deisz, W.D.C., Havelaar, A.H., Nagelkerke, N.J.D. & Henken, A.M. (2004). Risk factors for the presence of Campylobacter spp. in Dutch broiler flocks. *Preventive Veterinary Medicine*, **62**, 35–49.

Bratz, K., Bücker, R., Gölz, G., Zakrzewski, S.S., Janczyk, P., Nöckler, K. & Alter, T. (2013). Experimental infection of weaned piglets with *Campylobacter coli* - Excretion and translocation in a pig colonisation trial. *Veterinary Microbiology*, **162**, 136–143.

Breitenmoser, A., Fretz, R., Schmid, J., Besl, A. & Etter, R. (2011). Outbreak of acute gastroenteritis due to a washwater-contaminated water supply, Switzerland, 2008. *Journal of Water and Health*, **9**, 569–576.

Callicott, K.A., Frioriksdóttir, V., Reiersen, J., Lowman, R., Bisaillon, J.R., Gunnarsson, E., Berndtson, E., Hiett, K.L., Needleman, D.S. & Stern, N.J. (2006). Lack of evidence for vertical transmission of *Campylobacter* spp. in chickens. *Applied and Environmental Microbiology*, **72**, 5794–5798.

Carrique-Mas, J.J., Bryant, J.E., Cuong, N. V., Hoang, N.V.M., Campbell, J., Hoang, N. V., Dung, T.T.N., Duy, D.T., Hoa, N.T., Thompson, C., Hien, V. V., Phat, V. V., Farrar, J. & Baker, S. (2014). An epidemiological investigation of *Campylobacter* in pig and poultry farms in the Mekong delta of Vietnam. *Epidemiology and Infection*, **142**, 1425–1436.

Çelİk, E., Sağlam, A.G., Çelebİ, Ö. & Otlu, S. (2018). Isolation of *Arcobacter* spp . from domestic ducks and geese and identification of the recovered isolates by using molecular method. *Turkish Journal of Veterinary and Animal Sciences*, **42**, 1–6.

Červenka, L., Malíková, Z., Zachová, I. & Vytřasová, J. (2004). The effect of acetic acid, citric acid, and trisodium citrate in combination with different levels of water activity on the growth of Arcobacter butzleri in culture. *Folia Microbiologica*, **49**, 8–12.

Chau, M.L., Hartantyo, S.H.P., Yap, M., Kang, J.S.L., Aung, K.T., Gutiérrez, R.A., Ng, L.C., Tam, C.C. & Barkham, T. (2016). Diarrheagenic pathogens in adults attending a hospital in Singapore. *BMC Infectious Diseases*, **16**, 1–9.

Chokboonmongkol, C., Patchanee, P., Gölz, G., Zessin, K.H. & Alter, T. (2013). Prevalence, quantitative load, and antimicrobial resistance of *Campylobacter* spp. from broiler ceca and broiler skin samples in Thailand. *Poultry Science*, **92**, 462–467.

Clarke, M. (2018). WHO warms African countries about *Listeria* outbreak. [Internet document]. URL https://www.foodpoisonjournal.com/foodborne-illness-outbreaks/the-who-warns-african-countries-about-listeria-outbreak/ 06/10/2018.

Collado, L. & Figueras, M.J. (2011). Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clinical Microbiology Reviews*, **24**, 174–192.

Collado, L., Guarro, J. & Figueras, M.J. (2009). Prevalence of *Arcobacter* in Meat and Shellfish. *Journal of Food Protection*, **72**, 1102–1106.

Collado, L., Gutiérrez, M., González, M. & Fernández, H. (2013). Assessment of the prevalence and diversity of emergent *Campylobacteria* in human stool samples using a combination of traditional and molecular methods. *Diagnostic Microbiology and Infectious Disease*, **75**, 434–436.

Colles, F.M., Dingle, K.E., Cody, A.J. & Maiden, M.C.J. (2008a). Comparison of *Campylobacter* populations in wild geese with those in starlings and free-range poultry on the same farm. *Applied and Environmental Microbiology*, **74**, 3583–3590.

Colles, F.M., Jones, T.A., McCarthy, N.D., Sheppard, S.K., Cody, A.J., Dingle, K.E., Dawkins, M.S. & Maiden, M.C.J. (2008b). *Campylobacter* infection of broiler chickens in a free-range environment. *Environmental Microbiology*, **10**, 2042–2050.

Córdoba-Calderón, O., Redondo-Solano, M., Castro-Arias, E. & Arias-EchandI, M.L. (2017). *Arcobacter* Isolation from Minced Beef Samples in Costa Rica. *Journal of Food Protection*, **80**, 775–778.

CoşAnsu, S. & Ayhan, K. (2010). Effects of lactic and acetic acid treatments on campylobacter jejuni inoculated onto chicken leg and breast meat during storage at 4c and -18c. *Journal of Food Processing and Preservation*, **34**, 98–113.

Cox, A.N.A., Stern, N.J., Wilson, J.L., Musgrove, M.T. & Buhr, R.J. (2002). Isolation of *Campylobacter* spp. from semen samples of commercial broiler breeder roosters. *American Association of Avian Pathologists Stable*, **46**, 717–720.

Cuomo, A., Dipineto, L., Santaniello, A., Matteoli, G., Sarli, T., Vecchia, D. Della, Fioretti, A. & Menna, L.F. (2007). Detection of thermotolerant *Campylobacter* in ostriches (*Struthio camelus*) in Italy. *Veterinary Journal*, **174**, 439–441.

De Smet, S., Vandamme, P., Zutter, L. De, On, S.L.W., Douidah, L. & Houf, K. (2011a). *Arcobacter trophiarum* sp. nov., isolated from fattening pigs. *International Journal of Systematic and Evolutionary Microbiology*, **61**, 356–361.

De Smet, S., Zutter, L. De, Debruyne, L., Vangroenweghe, F., Vandamme, P. & Houf, K. (2011b). *Arcobacter* population dynamics in pigs on farrow-to-finish farms. *Applied and Environmental Microbiology*, **77**, 1732–1738.

De Smet, S., Zutter, L. De, Hende, J. Van & Houf, K. (2010). *Arcobacter* contamination on pre- and post-chilled bovine carcasses and in minced beef at retail. *Journal of Applied Microbiology*, **108**, 299–305.

Dekker, D., Eibach, D., Boahen, K.G., Akenten, C.W., Pfeifer, Y., Zautner, A.E., Mertens, E., Krumkamp, R., Jaeger, A., Flieger, A., Owusu-Dabo, E. & May, J. (2019). Fluoroquinolone-Resistant *Salmonella enterica*, *Campylobacter*

spp., and *Arcobacter butzleri* from Local and Imported Poultry Meat in Kumasi, Ghana. *Foodborne Pathogens and Disease*, **16**, 1–7.

Denis, M., Henrique, E., Chidaine, B., Tircot, A., Bougeard, S. & Fravalo, P. (2011). *Campylobacter* from sows in farrow-to-finish pig farms: Risk indicators and genetic diversity. *Veterinary Microbiology*, **154**, 163–170.

Deogratias, A.P., Mushi, M.F., Paterno, L., Tappe, D., Seni, J., Kabymera, R., Kidenya, B.R. & Mshana, S.E. (2014). Prevalence and determinants of *Campylobacter* infection among under five children with acute watery diarrhoea in Mwanza, North Tanzania. *Archives of Public Health*, **72**, 1–6.

Díaz-Sánchez, S., Moriones, A.M., Casas, F. & Höfle, U. (2004). Prevalence of *Escherichia coli*, *Salmonella* sp. and *Campylobacter* sp. in the intestinal flora of farm-reared, restocked and wild red-legged partridges (*Alectoris rufa*): Is restocking using farm-reared birds a risk? *European Journal of Wildlife Research*, **3**, 129–134.

Dipineto, L., Gargiulo, A., Bossa, L.M.D.L., Cuomo, A., Santaniello, A., Sensale, M., Menna, L.F. & Fioretti, A. (2008). Survey of thermotolerant *Campylobacter* in pheasant (*Phasianus colchicus*). *Italian Journal of Animal Science*, **7**, 401–403.

Duffy, L.L., Blackall, P.J., Cobbold, R.N. & Fegan, N. (2014). Quantitative effects of in-line operations on *Campylobacter* and *Escherichia coli* through two Australian broiler processing plants. *International Journal of Food Microbiology*, **188**, 128–134.

Duffy, L.L., Osmond-McLeod, M.J., Judy, J. & King, T. (2018). Investigation into the antibacterial activity of silver, zinc oxide and copper oxide nanoparticles against poultry-relevant isolates of Salmonella and Campylobacter. *Food Control*, **92**, 293–300.

ECDC & EFSA (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA Journal*, **13**, 1–231.

ECDC & EFSA (2017). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal*, **15**, 1–227.

EFSA (2008). Summary of annual report 2008. [Internet document]. URL https://www.efsa.europa.eu/. 10/05/2016.

Eifert, J.D., Castle, R.M., Pierson, F.W., Larsen, C.T. & Hackney, C.R. (2003). Comparison of sampling techniques for detection of *Arcobacter butzleri* from chickens. *Poultry Science*, **82**, 1898–1902.

Elmali, M. & Can, H.Y. (2016). Occurence and antimicrobial resistance of *Arcobacter* species in food and slaughterhouse samples. *Food Science and Technology (Campinas)*, **37**, 280–285.

Enokimoto, M., Kubo, M., Bozono, Y., Mieno, Y. & Misawa, N. (2007). Enumeration and identification of *Campylobacter* species in the liver and bile of slaughtered cattle. *International Journal of Food Microbiology*, **118**, 259–263.

Ertas, N., Dogruer, I.Y., Nulalan, Z.G.O., Guner, A. & Ulger, I. (2010). Prevalence of *Arcobacter* species in drinking water , spring water , and raw milk as determined by multiplex PCR, **73**, 2099–2102.

Euzeby, J.P. (2013). List of prokaryotic names withstanding in nomenclature genus *Campylobacter*. [Internet document]. URL www.bacterio.net/ 06/03/2017.

Euzeby, J.P. (2018). List of prokaryotic names withstanding in nomenclature genus *Campylobacter*. [Internet document]. URL www.bacterio.net/ 06/08/2018.

Evans, S.J. & Sayers, A.R. (2000). A longitudinal study of campylobacter infection of broiler flocks in Great Britain. *Preventive Veterinary Medicine*, **46**, 209–223.

Farmer, S., Keenan, A. & Vivancos, R. (2012). Food-borne *Campylobacter* outbreak in Liverpool associated with cross-contamination from chicken liver parfait: Implications for investigation of similar outbreaks. *Public Health*, **126**, 657–659.

Federighi, M. (2017) How to control *Campylobacter* in poultry farms?: An overview of the main strategies. In: *Poultry Science* (edited by M. Manafi) Pp. 115-132. Crotia: InTechOpen.

Fera, M.T., Camera, E. La, Carbone, M., Malara, D. & Pennisi, M.G. (2009). Pet cats as carriers of *Arcobacter* spp. in southern Italy. *Journal of Applied Microbiology*, **106**, 1661–1666.

Fera, M.T., Russo, G.T., Benedetto, A. Di, Camera, E. La, Orlando, A., Giandalia, A., Ruffa, V.F., Lanza, G., Lentini, V., Perdichizzi, G. & Cucinotta, D. (2010). High prevalence of *Arcobacter* carriage in older subjects with type 2 diabetes. *Journal of Biomedicine and Biotechnology*, **2010**, 1–7.

Fernandez, H., Villanueva, M.P., Mansilla, I., Gonzalez, M. & Latif, F. (2015). *Arcobacter butzleri* and *A. cryaerophilus* in human, animals and food sources, in southern Chile. *Brazilian Journal of Microbiology*, **46**, 145–147.

Ferreira, S., Fraqueza, M.J., Queiroz, J.A., Domingues, F.C. & Oleastro, M. (2013). Genetic diversity, antibiotic resistance and biofilm-forming ability of *Arcobacter butzleri* isolated from poultry and environment from a Portuguese slaughterhouse. *International Journal of Food Microbiology*, **162**, 82–88.

Figueroa, G., Troncoso, M., Ĺopez, C., Rivas, P. & Toro, M. (2009). Occurrence and enumeration of *Campylobacter* spp. during the processing of Chilean broilers. *BMC Microbiology*, **9**, 1–6.

Fong, T.T., Mansfield, L.S., Wilson, D.L., Schwab, D.J., Molloy, S.L. & Rose, J.B. (2007). Massive microbiological groundwater contamination associated with a waterborne outbreak in Lake Erie, South Bass Island, Ohio. *Environmental Health Perspectives*, **115**, 856–864.

Food Standards Australia New Zealand (2013). Assessment of microbiological hazards associated with the four main meat species. [Internet document]. URL www.foodstandards.gov.au/.../importedfoods/.../Beef%20and%20*Campylobacter*.pdf 15/02/2017.

Gaudreau, C., Helferty, M., Sylvestre, J.L., Allard, R., Pilon, P.A., Poisson, M. & Bekal, S. (2013). *Campylobacter coli*

outbreak in men who have sex with men, Quebec, Canada, 2010-2011. *Emerging Infectious Diseases*, **19**, 764–767.

Geissler, A., Carrillo, F.B., Swanson, K., Patrick, M.E., Fullerton, K.E., Bennet, C., Barret, K. & Mahon, B.E. (2017). Increasing *Campylobacter* infections, outbreaks, and antimicrobial resistance in the United States, 2004-2012. *Clinical infectious Diseases,* **65**, 1624–1631.

Ghafir, Y., China, B., Dierick, K., De Zutter, L. & Daube, G. (2008). Hygiene indicator microorganisms for selected pathogens on beef, pork, and poultry meats in Belgium. *Journal of Food Protection*, **71**, 35–45.

Giacometti, F., Lucchi, A., Francesco, A. Di, Delogu, M., Grilli, E., Guarniero, I., Stancampiano, L., Manfreda, G., Merialdi, G. & Serraino, A. (2015). *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii* circulation in a dairy farm and sources of milk contamination. *Applied and Environmental Microbiology*, **81**, 5055–5063.

Gifford, H., Paton, S., Cvitanovic, L., Mcmenamin, J. & Newton, C. (2012). *Arcobacter* species in diarrhoeal faeces from humans in New Zealand. *Journal of the New Zealand Medical Asoociation*, **125**, 17–25.

Gill, C.O. & Harris, L.M. (1982). Contamination of red-meat carcasses by *Campylobacter fetus* subsp. *jejuni*. *Applied and Environmental Microbiology*, **43**, 977–980.

Gölz, G., Kittler, S., Malakauskas, M. & Alter, T. (2018). Survival of *Campylobacter* in the Food Chain and the Environment. *Current Clinical Microbiology,* **5**, 126–134.

González, M., Mikkelä, A., Tuominen, P., Ranta, J., Hakkinen, M., Hänninen, M. & Llarena, A. (2016). Risk assessment of *Campylobacter* spp. in Finland. [Internet document]. URL https://www.evira.fi/globalassets/tietoa-evirasta/julkaisut/julkaisusarjat/elintarvikkeet/risk-assessment-of-*Campylobacter*-spp.pdf. 08/02/2017.

Gormley, F.J., Bailey, R.A., Watson, K.A., McAdam, J., Avendaño, S., Stanley, W.A. & Koerhuis, A.N.M. (2014). *Campylobacter* colonization and proliferation in the broiler chicken upon natural field challenge is not affected by the bird growth rate or breed. *Applied and Environmental Microbiology*, **80**, 6733–6738.

Gouws, P.A., Shange, N., & Hoffman, L.C. (2017). Microbial quality of springbok (*Antidorcas marsupialis*) meat in relation to harvesting and production process. In: *Game meat hygiene: Food safety and security* (edited by P. Paulsen, A, Bauer, & F.J.M. Smulders). Pp. 223-228. Netherlands: Wageningen Academic Publishers.

Grau, F.H. (1988). *Campylobacter-Jejuni* and *Campylobacter hyointestinalis* in the Intestinal tract and on the carcasses of calves and cattle. *Journal of Food Protection*, **51**, 857–861.

Griffiths, S.L., Salmon, R.L., Mason, B.W., Elliott, C., Thomas, D.R. & Davies, C. (2010). Using the internet for rapid investigation of an outbreak of diarrhoeal illness in mountain bikers. *Epidemiology and Infection*, **138**, 1704–1711.

Grove-White, D.H., Leatherbarrow, A.J.H., Cripps, P.J., Diggle, P.J. & French, N.P. (2014). Temporal and farm-management-associated variation in faecal pat prevalence of *Arcobacter* spp. in ruminants. *Epidemiology and Infection*, **142**, 861–870.

Gubbels, S.M., Kuhn, K.G., Larsson, J.T., Adelhardt, M., Engberg, J., Ingildsen, P., Hollesen, L.W., Muchitsch, S., Mølbak, K. & Ethelberg, S. (2012). A waterborne outbreak with a single clone of *Campylobacter jejuni* in the Danish town of Køge in May 2010. *Scandinavian Journal of Infectious Diseases*, **44**, 586–594.

Gwimi, P.B., Faleke, O.O., Salihu, M.D., Magaji, A.A., Abubakar, M.B., Nwankwo, I.O. & Ibitoye, E.B. (2015). Prevalence of *Campylobacter* species in fecal samples of pigs and humans from Zuru Kebbi State, Nigeria. *International Journal of One Health*, **1**, 1–5.

Habib, I., Zutter, L. De, Huffel, X. Van, Geeraerd, A.H. & Uyttendaele, M. (2012). Potential of *Escherichia coli* as a surrogate indicator for post-chill broiler carcasses with high *Campylobacter* counts. *Food Control*, **25**, 96–100.

Hald, T., Aspinall, W., Devleesschauwer, B., Cooke, R., Corrigan, T., Havelaar, A.H., Gibb, H.J., Torgerson, P.R., Kirk, M.D., Angulo, F.J., Lake, R.J., Speybroeck, N. & Hoffmann, S. (2016). World Health Organization estimates of the relative contributions of food to the burden of disease due to selected foodborne hazards: A structured expert elicitation. *PLoS ONE*, **11**, 1–35.

Hamidi, K. (2018). How do Rodents Play Role in Transmission of Foodborne Diseases? *Nutrition & Food Science International Journal*, **6**, 10–13.

Hara-Kudo, Y. & Takatori, K. (2011). Contamination level and ingestion dose of foodborne pathogens associated with infections. *Epidemiology and Infection*, **139**, 1505–1510.

Haruna, M., Sasaki, Y., Murakami, M., Mori, T., Asai, T., Ito, K. & Yamada, Y. (2013). Prevalence and Antimicrobial Resistance of *Campylobacter* Isolates from Beef Cattle and Pigs in Japan. *Journal of Veterinary Medical Science*, **75**, 625–628.

Hassan, A.K. (2017). Detection and Identification of *Arcobacter* species in poultry in Assiut Governorate, Upper Egypt. *Journal of Advanced Veterinary Research*, **7**, 53–58.

Hemsworth, S. & Pizer, B. (2006). Pet ownership in immunocompromised children-A review of the literature and survey of existing guidelines. *European Journal of Oncology Nursing*, **10**, 117–127.

Heredia, N. & García, S. (2018). Animals as sources of food-borne pathogens: A review. Animal *Nutrition Journal*, **2018**, 4–9.

Ho, H.T.K., Lipman, L.J.A. & Gaastra, W. (2008). The introduction of *Arcobacter* spp. in poultry slaughterhouses. *International Journal of Food Microbiology*, **125**, 223–229.

Ho, T.K.H., Lipman, L.J.A., Graaf-Van Bloois, L. Van Der, Bergen, M. Van & Gaastra, W. (2006). Potential routes of acquisition of *Arcobacter* species by piglets. *Veterinary Microbiology*, **114**, 123–133.

Horrocks, S.M., Anderson, R.C., Nisbet, D.J. & Ricke, S.C. (2009). Incidence and ecology of *Campylobacter jejuni* and *coli* in animals. *Anaerobe*, **15**, 18–25.

Houf, K. & Stephan, R. (2007). Isolation and characterization of the emerging foodborn pathogen *Arcobacter* from human stool. *Journal of Microbiological Methods*, **68**, 408–413.

Humphrey, S., Chaloner, G., Kemmett, K., Davidson, N., Williams, N., Kipar, A., Humphrey, T. & Wigley, P. (2014). Campylobacter jejuni is not merely a commensal in commercial broiler chickens and affects bird welfare. *mBio*, **5**, 1–7.

Humphrey, T., O'Brien, S. & Madsen, M. (2007). *Campylobacters* as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, **117**, 237–257.

Inns, T., Foster, K. & Gorton, R. (2010). Cohort study of a campylobacteriosis outbreak associated with chicken liver parfait, United Kingdom, june 2010. *Eurosurveillance*, **15**, 1–4.

Iwamoto, M., Ayers, T., Mahon, B.E. & Swerdlow, D.L. (2010). Epidemiology of seafood-associated infections in the United States. *Clinical Microbiology Reviews*, **23**, 399–411.

Janneth, R., Fernández, J. & Fernández, H. (2018). Report of first isolation of the zoonotic *Arcobacter* species from swine fecal samples in Ecuador, *Australian Journal of Veterinary* Sciences, **137**, 135–137.

Jiang, Z.D., Dupont, H.L., Brown, E.L., Nandy, R.K., Ramamurthy, T., Sinha, A., Ghosh, S., Guin, S., Gurleen, K., Rodrigues, S., Chen, J.J., McKenzie, R. & Steffen, R. (2010). Microbial etiology of travelers' diarrhea in Mexico, Guatemala, and India: Importance of enterotoxigenic bacteroides fragilis and *Arcobacter* Species. *Journal of Clinical Microbiology*, **48**, 1417–1419.

Johnsen, G., Zimmerman, K., Lindstedt, B.A., Vardund, T., Herikstad, H. & Kapperud, G. (2006). Intestinal carriage of *Campylobacter jejuni* and *Campylobacter coli* among cattle from South Western Norway and comparative genotyping of bovine and human isolates by amplified-fragment length polymorphism. *Acta Veterinaria Scandinavica*, **48**, 4–9.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, **28**, 687–720.

Kalupahana, R.S., Kottawatta, S.A. & Somarathne, S. (2018). Weather correlates of *Campylobacter* prevalence in broilers at slaughter under tropical conditions in Sri Lanka. *Epidemiology and infection*, **56**, 1–8.

Karagiannis, I., Sideroglou, T., Gkolfinopoulou, K., Tsouri, A., Lampousaki, D., Velonakis, E.N., Scoulica, E. V., Mellou, K., Panagiotopoulos, T. & Bonovas, S. (2010). A waterborne *Campylobacter jejuni* outbreak on a Greek island. *Epidemiology and Infection*, **138**, 1726–1734.

Karikari, A.B., Obiri-Danso, K., Frimpong, E.H. & Krogfelt, K.A. (2017). Antibiotic Resistance of *Campylobacter* Recovered from Faeces and carcasses of healthy livestock. *BioMed Research International*, **2017**, 1–9.

Kayman, T., Abay, S., Hizlisoy, H., Ibrahim Atabay, H., Serdar Diker, K. & Aydin, F. (2012). Emerging pathogen *Arcobacter* spp. in acute gastroenteritis: Molecular identification, antibiotic susceptibilities and genotyping of the isolated *Arcobacter*s. *Journal of Medical Microbiology*, **61**, 1439–1444.

Keener, K.M., Bashor, M.P., Curtis, P.A., Sheldon, B.W. & Kathariou, S. (2004). Comprehensive review of *Campylobacter* and poultry processing. *Reviews in Food Science and Food Safety*, **3**, 105–116.

Kempf, I., Kerouanton, A., Bougeard, S., Nagard, B., Rose, V., Mourand, G., Osterberg, J., Denis, M. & Bengtsson, B.O. (2017). *Campylobacter coli* in organic and conventional pig production in France and Sweden: Prevalence and antimicrobial resistance. *Frontiers in Microbiology*, **8**, 1–9.

Khoshbakht, R., Tabatabaei, M., Shirzad Aski, H. & Seifi, S. (2014). Occurrence of *Arcobacter* in Iranian poultry and slaughterhouse samples implicates contamination by processing equipment and procedures. *British Poultry Science*, **55**, 732–736.

Kim, N.H., Park, S.M., Kim, H.W., Cho, T.J., Kim, S.H., Choi, C. & Rhee, M.S. (2018). Prevalence of pathogenic *Arcobacter* species in South Korea: Comparison of two protocols for isolating the bacteria from foods and examination of nine putative virulence genes. *Food Microbiology*, **78**, 18–24.

King, T., Osmond-McLeod, M.J. & Duffy, L.L. (2018). Nanotechnology in the food sector and potential applications for the poultry industry. *Trends in Food Science and Technology*, **72**, 62–73.

Kist, M. (2002). Lebensmittelbedingte Infektionen durch *Campylobacter*. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz*, **45**, 497–506.

Komba, E.V.G., Mdegela, R.H., Msoffe, P.L.M. & Ingmer, H. (2013). Human and animal campylobacteriosis in Tanzania: A review. *Tanzania Journal of Health Research*, **15**, 1–13.

Kopilovi, B., Ucakar, V., Koren, N., Krek, M. & Kraigher, A. (2008). Outbreak of acute gastroenteritis in coastal area in Slovenia. [Internet document]. URL https://www.eurosurveillance.org/content/10.2807/ese.13.34.18957-en 10/10/2018.

Korczak, B.M., Stieber, R., Emler, S., Burnens, A.P., Frey, J. & Kuhnert, P. (2006). Genetic relatedness within the genus *Campylobacter* inferred from rpoB sequences. *International Journal of Systematic and Evolutionary Microbiology*, **56**, 937–945.

Kownhar, H., Shankar, E.M., Rajan, R., Vengatesan, A. & Rao, U.A. (2007). Prevalence of *Campylobacter jejuni* and enteric bacterial pathogens among hospitalized HIV infected versus non-HIV infected patients with diarrhoea in southern India. *Scandinavian Journal of Infectious Diseases*, **39**, 862–866.

Lastovica, A.J. & Roux, E. (2000). Efficient isolation of *Campylobacteria* from stools efficient isolation of *Campylobacteria* from Stools, **38**, 2798–2800.

Lastovica, A.J. (2006). Emerging *Campylobacter* spp.: The tip of the iceberg. *Clinical Microbiology Newsletter*, **28**, 49–56.

Lastovica, A.J., On, L.W. & Zhang, L. (2014). The Family *Campylobacteraceae*. In: *The prokaryotes-Deltaproteobacteria and Epsilonproteobacteria* (edited by E. Rosenberg *et al.*). Pp. 308 – 326. Berlin: Springer-Verlag.

Lazou, T., Fragkou, F., Gelasakis, A., Dovas, C., Soultos, N., Rallis, T., Iossifidou, E., Unit, M. & Clinic, A. (2017). Prevalence, antimicrobial resistance and risk factors for *Campylobacter* colonising dogs and cats in Greece, *Bulgarian Journal of Veterinary Medicine*, **20**, 244–254.

Leblanc Maridor, M., Denis, M., Lalande, F., Beaurepaire, B., Cariolet, R., Fravalo, P., Federighi, M., Seegers, H. & Belloc, C. (2008). Experimental infection of specific pathogen-free pigs with *Campylobacter*: Excretion in faeces and transmission to non-inoculated pigs. *Veterinary Microbiology*, **131**, 309–317.

Levican, A., Collado, L. & Figueras, M.J. (2013). *Arcobacter cloacae* sp. nov. and *Arcobacter suis* sp. nov., two new species isolated from food and sewage. *Systematic and Applied Microbiology*, **36**, 22–27.

Levican, A., Rubio-Arcos, S., Martinez-Murcia, A., Collado, L. & Figueras, M.J. (2015). *Arcobacter ebronensis* sp. nov. and *Arcobacter aquimarinus* sp. nov., two new species isolated from marine environment. *Systematic and Applied Microbiology*, **38**, 30–35.

Levin, R.E. (2007). *Campylobacter jejuni*: A review of its characteristics, pathogenicity, ecology, distribution, subspecies characterization and molecular methods of detection. *Food Biotechnology*, **21**, 271–347.

Lindmark, H., Diedrich, I.C., Andersson, L., Lindqvist, R. & Engvall, E.O. (2006). Distribution of *Campylobacter* genotypes on broilers during slaughter. *Journal of food protection*, **69**, 2902–2907.

Ling, Y.E., Aziz, S.A. & Abu, J. (2011). Occurrence of *Campylobacter* and *Salmonella* spp. in Ostrich. *J. Vet. Malaysia*, **24**, 6–8.

Lipman, L., Ho, H. & Gaastra, W. (2008). The presence of *Arcobacter* species in breeding hens and eggs from these hens. *Poultry Science*, **87**, 2404–2407.

Little, C.L.Ã., Richardson, J.F., Owen, R.J., Pinna, E. De & Threlfall, E.J. (2008). *Campylobacter* and *Salmonella* in raw red meats in the United Kingdom: Prevalence, characterization and antimicrobial resistance pattern, 2003 – 2005. *Food Microbiology*, **25**, 538–543.

Louwen, R., van Baarlen, P., van Vliet, A.H.M., van Belkum, A., Hays, J.P. & Endtz, H.P. (2012). *Campylobacter* bacteraemia: A rare and under-reported event? *European Journal of Microbiology and Immunology*, **2**, 76–87.

Luber, P., Brynestad, S., Topsch, D., Scherer, K. & Bartelt, E. (2006). Quantification of *Campylobacter* species cross-contamination during handling of contaminated fresh chicken parts in kitchens. *Applied and Environmental Microbiology*, **72**, 66–70.

Luque, I., Tarradas, C., Herrera-León, S., Gómez-Laguna, J., Hernández, M., Maldonado, A., Astorga, R.J., Morales-Partera, A.M. & Cardoso-Toset, F. (2018). Prevalence and diversity of *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes* in two free-range pig slaughterhouses. *Food Control*, **92**, 208–215.

Mabote, K.I., Mbewe, M. & Ateba, C.N. (2011). Prevalence of *Campylobacter* contamination in fresh chicken meat and milk obtained from markets in the North-West Province, South Africa. *Journal of Human Ecology*, **36**, 23–28.

Maramski, A. (2012). Prevalence of *Campylobacter* spp. in pig Slaughter carcasses during the processing. *Trakia Journal of Sciences*, **10**, 62–67.

Mason, J., Iturriza-Gomara, M., O'Brien, S.J., Ngwira, B.M., Dove, W., Maiden, M.C.J. & Cunliffe, N.A. (2013). Campylobacter Infection in Children in Malawi Is Common and Is Frequently Associated with Enteric Virus Co-Infections. *PLoS ONE*, **8**, 1–5.

Merga, J.Y., Williams, N.J., Miller, W.G., Leatherbarrow, A.J.H., Bennett, M., Hall, N., Ashelford, K.E. & Winstanley, C. (2013). Exploring the diversity of *Arcobacter butzleri* from cattle in the UK using MLST and whole genome sequencing. *PLoS ONE*, **8**, 1–12.

Milnes, A.S., Stewart, I., Clifton-Hadley, F.A., Davies, R.H., Newell, D.G., Sayers, A.R., Cheasty, T., Cassar, C., Ridley, A., Cook, A.J.C., Evans, S.J., Teale, C.J., Smith, R.P., Mcnally, A., Toszeghy, M., Futter, R., Kay, A. & Paiba, G.A. (2008). Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. *Epidemiology and Infection*, **136**, 739–751.

Mohan, H.V., Rathore, R.S., Dhama, K., Ramees, T.P., Patya, A., Bagalko, P.S., Wani, M.Y., Bhilegaonkar, K.N. & Kumar, A. (2014). Prevalence of *Arcobacter* spp. in humans, animals and foods of animal origin in India based on cultural isolation, antibiogram, PCR and multiplex PCR detection. *Asian Journal of Animal and Veterinary Advances*, **9**, 452-466.

Møller-Stray, J., Eriksen, H.M., Bruheim, T., Kapperud, G., Lindstedt, B.A., Skeie, Å., Sunde, M., Urdahl, A.M., Øygard, B. & Vold, L. (2012). Two outbreaks of diarrhoea in nurseries in Norway after farm visits, April to May 2009. *Eurosurveillance*, **17**, 1–7.

Moriarty, E.M., McEwan, N., Mackenzie, M., Karki, N., Sinton, L.W. & Wood, D.R. (2011). Incidence and prevalence of microbial indicators and pathogens in ovine faeces in New Zealand. *New Zealand Journal of Agricultural Research*, **54**, 71–81.

Mpalang, R.K., Boreux, R., Melin, P., Akir Ni Bitiang, K., Daube, G. & Mol, P. De. (2014). Prevalence of Campylobacter among goats and retail goat meat in Congo. *Journal of Infection in Developing Countries*, **8**, 168–175.

Nakari, U.M., Huovinen, E., Kuusi, M. & Siitonen, A. (2010). Population-based surveillance study of *Campylobacter* infections in Finland. *Epidemiology and infection*, **138**, 1712–8.

Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., Logt, P. van der, Fazil, A., Christensen, B., Katsma, E., Borck, B. & Havelaar, A. (2009). A comparison of risk assessments on *Campylobacter* in broiler meat. *International Journal of Food Microbiology*, **129**, 107–123.

Nielsen, H.L., Ejlertsen, T., Engberg, J. & Nielsen, H. (2013). High incidence of *Campylobacter concisus* in gastroenteritis in North Jutland, Denmark: A population-based study. *Clinical Microbiology and Infection*, **19**, 445–450.

On, S.L.W. (2001). Taxonomy of *Campylobacter*, *Arcobacter*, *Helicobacter* and related bacteria: Current status, future prospects and immediate concerns. *Journal of Applied Microbiology*, **90**, 1S–15S.

On, S.L.W., Miller, W.G., Houf, K., Fox, J.G. & Vandamme, P. (2017). Minimal standards for describing new species belonging to the families *Campylobacteraceae* and *Helicobacteraceae*: *Campylobacter*, *Arcobacter, Helicobacter* and *Wolinella* spp. *International Journal of Systematic and Evolutionary Microbiology*, **67**, 5296–5311.

Oporto, B., Esteban, J.I., Aduriz, G., Juste, R.A. & Hurtado, A. (2007). Prevalence and strain diversity of thermophilic *Campylobacters* in cattle, sheep and swine farms. *Journal of Applied Microbiology*, **103**, 977–984.

Osiriphun, S., Iamtaweejaloen, P., Kooprasertying, P., Koetsinchai, W., Tuitemwong, K., Erickson, L.E. & Tuitemwong, P. (2011). Exposure assessment and process sensitivity analysis of the contamination of *Campylobacter* in poultry products. *Poultry Science*, **90**, 1562–1573.

Oyarzabal, O.A., Donald, E.C. & Hoerrb, F.J. (1995). Incidence of *Campylobacters* in the Intestine of avian Species in Alabama. *Avian Diseases*, **39**, 147–151.

Pacholewicz, E., Swart, A. & Havelaar, A.H. (2016). Explanatory variables associated with *Campylobacter* and *Escherichia coli* concentrations on broiler chicken carcasses during processing in two slaughterhouses. *Journal of Food Protection*, **79**, 2038-2047.

Padungtod, P., Kaneene, J.B. & P., P. (2005). *Campylobacter* in food animals and humans in Northern Thailand. *Journal of Food Protection*, **68**, 2519–2526.

Park, S.F. (2002). The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *International Journal of Food Microbiology*, **74**, 177–188.

Parry, A., Fearnley, E. & Denehy, E. (2012). 'Surprise': Outbreak of *Campylobacter* infection associated with chicken liver pâté at a surprise birthday party, Adelaide, Australia, 2012. *Western Pacific Surveillance and Response Journal*, **3**, 16–19.

Patyal, A., Rathore, R.S., Mohan, H. V., Dhama, K. & Kumar, A. (2011). Prevalence of *Arcobacter* spp. in humans, animals and foods of animal origin Including sea food from India. *Transboundary and Emerging Diseases*, **58**, 402–410.

Pearce, R.A., Wallace, F.M., Call, J.E., Dudley, R.L., Oser, A., Yoder, L., Sheridan, J.J. & Luchansky, J.B. (2003). Prevalence of *Campylobacter* within a swine slaughter and processing facility. *Journal of Food Protection*, **66**, 1550–1556.

Pejchalova, M., Zabcikova, S., Silhova, L., Silha, D., Brozkova, I. & Haslova, M. (2016). Presence of *Arcobacter* species in pet cats and dogs in the Czech Republic. *Veterinarni Medicina*, **61**, 449–455.

Percivalle, E., Monzillo, V., Pauletto, A., Marone, P. & Imberti, R. (2016). Colistin inhibits E. coli O157:H7 Shiga-like toxin release, binds endotoxins and protects Vero cells. *New Microbiologica*, **39**, 119–123.

Perko-Mäkelä, P., Isohanni, P., Katzav, M., Lund, M., Hänninen, M.L. & Lyhs, U. (2009). A longitudinal study of *Campylobacter* distribution in a turkey production chain. *Acta Veterinaria Scandinavica*, **51**, 1–10.

Petersen, R.F., Harrington, C.S., Kortegaard, H.E. & On, S.L.W. (2007). A PCR-DGGE method for detection and identification of *Campylobacter, Helicobacter, Arcobacter* and related *Epsilobacteria* and its application to saliva samples from humans and domestic pets. *Journal of Applied Microbiology*, **103**, 2601–2615.

Peyrat, M.B., Soumet, C., Maris, P. & Sanders, P. (2008a). Recovery of *Campylobacter jejun*i from surfaces of poultry slaughterhouses after cleaning and disinfection procedures: Analysis of a potential source of carcass contamination. *International Journal of Food Microbiology*, **124**, 188–194.

Peyrat, M.B., Soumet, C., Maris, P. & Sanders, P. (2008b). Phenotypes and genotypes of *Campylobacter* strains isolated after cleaning and disinfection in poultry slaughterhouses. *Veterinary Microbiology,* **128**, 313–326.

Platts-mills, J.A. & Kosek, M. (2015). Update on the burden of *Campylobacter* in developing countries. *Current Opinion in Infectious Diseases*, **27**, 444–450.

Prince Milton, A.A., Agarwal, R.K., Priya, G.B., Saminathan, M., Aravind, M., Reddy, A., Athira, C.K., Anjay, Ramees, T.P., Dhama, K., Sharma, A.K. & Kumar, A. (2017). Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in captive wildlife species in India. *Iranian Journal of Veterinary Research*, **18**, 177–182.

Prouzet-Mauléon, V., Labadi, L., Bouges, N., Ménard, A. & Mégraud, F. (2006). *Arcobacter butzleri*: Underestimated enteropathogen. *Emerging Infectious Diseases*, **12**, 307–309.

Public Health Ontario. (2014). Monthly infectious diseases surveillance report. [Internet document]. URL http://www.publichealthontario.ca/en/DataAndAnalytics/Documents/PHO_Monthly_Infectious_Diseases_Surveillance_Report_-_February_2014.pdf 15/03/2018.

Ramees, T.P., Dhama, K., Karthik, K., Rathore, R.S., Kumar, A., Saminathan, M., Tiwari, R., Malik, Y.S. & Singh, R.K. (2017). *Arcobacter*: An emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control - A comprehensive review. *Veterinary Quarterly*, **37**, 136–161.

Ramonaitė, S., Rokaitytė, A., Tamulevičienė, E., Malakauskas, A., Alter, T. & Malakauskas, M. (2013). Prevalence, quantitative load and genetic diversity of *Campylobacter* spp. in dairy cattle herds in Lithuania. *Acta Veterinaria Scandinavica*, **55**, 87.

Räsänen, S., Lappalainen, S., Kaikkonen, S., Hämäläinen, M., Salminen, M. & Vesikari, T. (2010). Mixed viral infections causing acute gastroenteritis in children in a waterborne outbreak. *Epidemiology and Infection*, **138**, 1227–1234.

Rasschaert, G., Houf, K., Hende, J. Van & Zutter, L. De. (2006). *Campylobacter* contamination during poultry slaughter in Belgium. *Journal of food protection*, **69**, 27–33.

Reich, F., Atanassova, V., Haunhorst, E. & Klein, G. (2008). The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter. International Journal of Food Microbiology*, **127**, 116–120.

Rice, E.W., Rodgers, M.R., Wesley, I. V., Johnson, C.H. & Tanner, S.A. (1999). Isolation of *Arcobacter butzleri* from ground water. *Letters in Applied Microbiology*, **28**, 31–35.

Ridley, A.M., Morris, V.K., Cawthraw, S.A., Ellis-Iversen, J., Harris, J.A., Kennedy, E.M., Newell, D.G. & Allen, V.M. (2011). Longitudinal molecular epidemiological study of thermophilic *Campylobacters* on one conventional broiler chicken farm. *Applied and Environmental Microbiology*, **77**, 98–107.

Roccato, A., Mancin, M., Barco, L., Cibin, V., Antonello, K., Cocola, F. & Ricci, A. (2018). Usefulness of indicator bacteria as potential marker of *Campylobacter* contamination in broiler carcasses. *International Journal of Food Microbiology*, **276**, 63–70.

Rosenquist, H., Sommer, H.M., Nielsen, N.L. & Christensen, B.B. (2006). The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter. International Journal of Food Microbiology*, **108**, 226–232.

Rotariu, O., Dallas, J.F., Ogden, I.D., MacRae, M., Sheppard, S.K., Maiden, M.C.J., Gormley, F.J., Forbes, K.J. & Strachan, N.J.C. (2009). Spatiotemporal homogeneity of *Campylobacter* subtypes from cattle and sheep across North Eastern and South Western Scotland. *Applied and Environmental Microbiology*, **75**, 6275–6281.

Sagoo, S.K., Little, C.L., Allen, G., Williamson, K. & Grant, K. a. (2007). Microbiological safety of retail vacuum-packed and modified-atmosphere-packed cooked meats at end of shelf life. *Journal of food protection*, **70**, 943–951.

Sahin, O., Kassem, I.I., Shen, Z., Lin, J., Rajashekara, G. & Zhang, Q. (2015). *Campylobacter* in poultry: Ecology and Potential Interventions. *Avian Diseases*, **59**, 185–200.

Sahin, O., Kobalka, P. & Zhang, Q. (2003). Detection and survival of *Campylobacter* in chicken eggs. *Journal of Applied Microbiology*, **95**, 1070–1079.

Samie, A., Obi, C.L., Barrett, L.J., Powell, S.M. & Guerrant, R.L. (2007). Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: Studies using molecular diagnostic methods. *Journal of Infection*, **54**, 558–566.

Sasi Jyothsna, T.S., Rahul, K., Ramaprasad, E.V.V., Sasikala, C. & Ramana, C. V. (2013). *Arcobacter anaerophilus* sp. nov., isolated from an estuarine sediment and emended description of the genus *Arcobacter. International Journal of Systematic and Evolutionary Microbiology*, **63**, 4619–4625.

Schets, F.M., Jacobs-Reitsma, W.F., van der Plaats, R.Q.J., Heer, L.K., van Hoek, A.H.A.M., Hamidjaja, R.A., de Roda Husman, A.M. & Blaak, H. (2017). Prevalence and types of *Campylobacter* on poultry farms and in their direct environment. *Journal of Water and Health*, **15**, 849–862.

Seguino, A., & Chintoan-Uta, C. (2017). *Campylobacter* spp. carriage in wild game pheasants (*Phasianus colchicus*) in Scotland and its relevance to public health. In: *Game meat hygiene: Food safety and security* (edited by P. Paulsen, A, Bauer & F.J.M. Smulders). Pp. 228-230. Wageningen: Academic Publishers.

Seliwiorstow, T., Baré, J., Damme, I. Van, Uyttendaele, M. & Zutter, L. De. (2015). *Campylobacter* carcass contamination throughout the slaughter process of *Campylobacter*-positive broiler batches. *International Journal of Food Microbiology*, **194**, 25–31.

Semaan, E.H., Dib, H., Mrad, R., Chami, C. & Jalkh, R. (2014). Dynamic of *Campylobacter* species contamination along a poultry slaughtering chain. *Italian Journal of Food Safety*, **3**, 185–187.

Shirzad Aski, H., Tabatabaei, M., Khoshbakht, R. & Raeisi, M. (2016). Occurrence and antimicrobial resistance of emergent *Arcobacter* spp. isolated from cattle and sheep in Iran. *Comparative Immunology, Microbiology and Infectious Diseases*, **44**, 37–40.

Shobo, C.O., Bester, L.A., Baijnath, S., Somboro, A.M., Peer, A.K.C. & Essack, S.Y. (2016). Antibiotic resistance profiles of *Campylobacter* species in the South Africa private health care sector. *Journal of Infection in Developing Countries*, **10**, 1214–1221.

Sibanda, N., McKenna, A., Richmond, A., Ricke, S.C., Callaway, T., Stratakos, A.C., Gundogdu, O.& C. (2004). A Review of the Effect of Management Practices on Campylobacter Prevalence in Poultry Farms. *International Journal of Poultry Science*, **3**, 129–134.

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P.A. & Teixeira, P. (2011). *Campylobacter* spp. as a foodborne pathogen: A review. *Frontiers in Microbiology*, **2**, 1–12.

Skarp, C.P.A., Hänninen, M.L. & Rautelin, H.I.K. (2016). Campylobacteriosis: The role of poultry meat. *Clinical Microbiology and Infection*, **22**, 103–109.

Son, I., Englen, M.D., Berrang, M.E., Fedorka-Cray, P.J. & Harrison, M.A. (2007). Prevalence of Arcobacter and Campylobacter on broiler carcasses during processing. *International Journal of Food Microbiology*, **113**, 16–22.

Sproston, E.L., Ogden, I.D., MacRae, M., Dallas, J.F., Sheppard, S.K., Cody, A.J., Colles, F.M., Wilson, M.J., Forbes, K.J. & Strachan, N.J.C. (2011). Temporal variation and host association in the *Campylobacter* population in a longitudinal ruminant farm study. *Applied and Environmental Microbiology*, **77**, 6579–6586.

Stahl, M., Butcher, J. & Stintzi, A. (2012). Nutrient acquisition and metabolism by *Campylobacter jejuni. Frontiers in cellular and infection microbiology*, **2**, 1-5.

Stanley, K. & Jones, K. (2003). Cattle and sheep farms as reservoirs of *Campylobacter. Journal of applied microbiology*, **94**, 104S–113S.

Stanley, K.N., Wallace, J.S., Currie, J.E., Diggle, P.J. & Jones, K. (1998). The seasonal variation of thermophilic *Campylobacters* in beef cattle, dairy cattle and calves. *Journal of Applied Microbiology*, **85**, 472–480.

Stephens, C.P., On, S.L.W. & Gibson, J.A. (1998). An outbreak of infectious hepatitis in commercially reared ostriches associated with *Campylobacter coli* and *Campylobacter jejuni. Veterinary Microbiology,* **61**, 183–190.

Stuart, T.L., Sandhu, J., Stirling, R., Corder, J., Ellis, A., Misa, P., Goh, S., Wong, B., Martiquet, P., Hoang, L. & Galanis, E. (2010). Campylobacteriosis outbreak associated with ingestion of mud during a mountain bike race. *Epidemiology and Infection*, **138**, 1695–1703.

Tanaka, R., Cleenwerck, I., Mizutani, Y., Iehata, S., Bossier, P. & Vandamme, P. (2017). Arcobacter haliotis sp. nov., isolated from abalone species Haliotis gigantea. *International Journal of Systematic and Evolutionary Microbiology*, **67**, 3050–3056.

Umar, S., Maiyah, A.T. & Mushtaq, A. (2016). *Campylobacter* infections in poultry: Update on challenges and potential immune interventions. *World's Poultry Science Journal*, **72**, 381–390.

Van Driessche, E. & Houf, K. (2007a). Discrepancy between the occurrence of *Arcobacter* in chickens and broiler carcass contamination. *Poultry Science*, **86**, 744–751.

Van Driessche, E. & Houf, K. (2007b). Characterization of the *Arcobacter* contamination on Belgian pork carcasses and raw retail pork. *International Journal of Food Microbiology*, **118**, 20–26.

Van Driessche, E., Houf, K., Vangroenweghe, F., Nollet, N., Zutter, L. De, Vandamme, P. & Hoof, J. Van. (2004). Occurrence and strain diversity of *Arcobacter* species isolated from healthy Belgian pigs. *Research in Microbiology*, **155**, 662–666.

Van Driessche, E., Houf, K., Vangroenweghe, F., Zutter, L. De & Hoof, J. Van. (2005). Prevalence, enumeration and strain variation of *Arcobacter* species in the faeces of healthy cattle in Belgium. *Veterinary Microbiology*, **105**, 149–154.

Vandamme, P., Pugina, P., Benzi, G., Etterijck, R. Van, Vlaes, L., Kersters, K., Butzler, J.P., Lior, H. & Lauwers, S. (1992). Outbreak of recurrent abdominal cramps associated with *Arcobacter butzleri* in an Italian school. *Journal of Clinical Microbiology*, **30**, 2335–2337.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N., Zissis, G. & Butzler, J. (2004). *Arcobacter* species in humans. *Emerging Infectious Diseases*, **10**, 1863-1867.

Vandeplas, S., Marcq, C., Dauphin, R.D., Beckers, Y., Thonart, P. & Théwis, A. (2008). Contamination of poultry flocks by the human pathogen *Campylobacter* spp. and strategies to reduce its prevalence at the farm level, **12**, 317–334.

Wagenaar, J. a, Mevius, D.J. & Havelaar, a H. (2006). *Campylobacter* in primary animal production and control strategies to reduce the burden of human campylobacteriosis. *Revue scientifique et technique (International Office of Epizootics)*, **25**, 581–94.

Wagenaar, J.A., French, N.P. & Havelaar, A.H. (2013). Preventing *Campylobacter* at the source: Why is it so difficult? *Clinical Infectious Diseases*, **57**, 1600–1606.

Webb, A.L., Boras, V.F., Kruczkiewicz, P., Selinger, L.B., Taboada, E.N. & Inglis, G.D. (2016). Comparative detection and quantification of *Arcobacter butzleri* in stools from diarrheic and nondiarrheic people in South Western Alberta, Canada. *Journal of Clinical Microbiology*, **54**, 1082–1088.

Weber, R., Auerbach, M., Jung, A. & Glünder, G. (2014). *Campylobacter* infections in four poultry species in respect of frequency, onset of infection and seasonality. *Berliner und Münchener tier ärztliche Wochenschrift*, **127**, 257–266.

Wensley, A. & Coole, L. (2013). Cohort study of a dual-pathogen point source outbreak associated with the consumption of chicken liver pâté, UK, October 2009. *Journal of Public Health (United Kingdom)*, **35**, 585–589.

Wesley, I. V & Baetz, A.L. (1996). Natural and experimental infections of *Arcobacter* in poultry. *Poultry Science*, **78**, 536–545.

Whyte, P., Collins, J.D., McGill, K., Monahan, C. & O'Mahony, H. (2001). The effect of transportation stress on excretion rates of *Campylobacter*s in market-age broilers. *Poultry Science*, **80**, 817–820.

Whyte, P., Mcgill, K., Cowley, D., Madden, R.H., Moran, L. & Scates, P. (2004). Occurrence of *Campylobacter* in retail foods in Ireland. *International Journal of Food Microbiology*, **95**, 111–118.

Wieczorek, K., Denis, E. & Osek, J. (2009). Occurrence of four major food-borne pathogens in cattle slaughtered in Poland. *Bulletin of the Veterinary Institute in Pulawy*, **53**, 439–444.

Workman, S.N., Mathison, G.E., Marc, C. & Lavoie, M.C. (2005). Pet dogs and chicken meat as reservoirs of *Campylobacter* spp. in Barbados's pet dogs and chicken meat as reservoirs of *Campylobacter* spp. in Barbados. *Journal of clinical microbiology*, **43**, 2642–2650.

World Health Organisation. (2012). The global view of campylobacteriosis. [Internet document]. URL www.who.int/iris/bitstream/10665/80751/1/9789241564601_eng.pdf IV. 18/06/2017.

Yu, J.H., Kim, N.Y., Cho, N.G., Kim, J.H., Kang, Y.A. & Lee, H.G. (2010). Epidemiology of Campylobacter jejuni outbreak in a middle school in Incheon, Korea. *Journal of Korean Medical Science*, **25**, 1595–1600.

Ziprin, R.L., Young, C.R., Stanker, L.H., Hume, M.E., Konkel, M.E. & Konkelb, M.E. (1999). The Absence of Cecal colonization of chicks by a mutant of *Campylobacter jejuni* not expressing bacterial fibronectin-binding protein. *American Association of Avian Pathologists*, **43**, 586–589.

CHAPTER 3

The onset of *Campylobacter* spp. and *Arcobacter* spp. colonisation in ostriches from South Africa

Abstract

The onset and prevalence of *Campylobacter* and *Arcobacter* species in ostrich chicks and slaughter-aged ostriches reared on farms located in Oudtshoorn (Western Cape) was investigated. Ostrich chicks were reared artificially and naturally at a chick rearing facility until the age of 12 weeks. Artificially and naturally reared chicks were sacrificed (n = 3 per treatment per sampling date) on the 2nd, 4th, 6th and 12th week of life. Cohorts that belonged to same batch as the sacrificed ostrich chicks were moved to a different farm and reared until the age of slaughter. Cloacal swabs were obtained from slaughter-aged ostriches at the age of 10 (n = 30) and 12 (n = 30) months. Non-selective (Cape Town protocol used for both *Campylobacter* spp. and *Arcobacter* spp.) and selective methods (ISO 10272-1:2006 and selective *Arcobacter* spp. method) were used for detection. The ISO 10272-1:2006 method detected *Campylobacter* spp. (*C. jejuni*) in artificially reared chicks, on the 12th week, the Cape Town protocol could not detect *Campylobacter* spp. A persistent presence of *Arcobacter* (*A. skirrowii*) was detected from the 2nd until 12th week of life for both artificially and naturally reared ostrich chicks with both the selective *Arcobacter* spp. method and the Cape Town protocol. Additionally, *Arcobacter* spp. (*A. skirrowii*) and *Campylobacter* spp. (*C. jejuni*) were isolated from 56-70% of slaughter-aged ostriches sampled. Overall, this study is an indication that in a typical farm *Campylobacter* spp. and *Arcobacter* spp. can infect and prevail in ostrich chicks and slaughter aged ostriches.

3.1 Introduction

Species that belong to the *Campylobacteraceae* family can be a risk to human health. Currently, *Campylobacter* species are known to induce a gastrointestinal infection known as campylobacteriosis, which is typically characterised as a self-limiting gastroenteritis syndrome manifested as abdominal cramps, pain, nausea, fever and diarrhoea (Altekruse & Tollefson, 2003; Kaakoush *et al.*, 2015). In order to induce campylobacteriosis, as little as 360 to 800 *Campylobacter* cells need to be ingested (Robert *et al.*, 1988; Keener *et al.*, 2004). The epidemiology of *Campylobacter* infections has been researched extensively, and findings suggests that there is an increase in *Campylobacter*-related illnesses in developed and developing countries. In fact, campylobacteriosis cases have increased in developed countries such as Australia, Europe, North and Central America (Kaakoush *et al.*, 2015; Heredia & García, 2018) and the incidence rate has been reported to range from 1.3 to 197 per a population size of 100 000 (European Centre for Disease Prevention and Control (ECDC) & European Food Safety Authority (EFSA), 2015). Even though it is not mandatory to report campylobacteriosis cases in developing countries, research has reported an incidence rate of 2.8 to 10.2%, additionally developing countries, mainly African and South East Asian countries, bear almost half of the global burden of *Campylobacter* (Percivalle *et al.*, 2016).

Arcobacter species are reported to be similar to *Campylobacter* species, however, *Arcobacter* species have the ability to survive in aerobic conditions and grow at lower temperatures (Sasi Jyothsna *et al.*, 2013). Within the realm of food safety, there is a lack of research focus towards *Arcobacter* species even though the International Commission on Microbiological specification for Food (ICMSF) deemed it a hazard to human health, in the year 2002 (Vandenberg *et al.*, 2004). Similar to *Campylobacter* spp., the ingestion of *Arcobacter* spp. could result in gastrointestinal infections. From research that compares the genome of *Arcobacter* spp. to *Campylobacter* spp. it is clear that these species might share the same virulence factors (Barboza *et al.*, 2017), possibly indicating that the infective dose of *Arcobacter* spp. could also be low. As it stands, gastrointestinal infections caused by *Arcobacter* spp. are mostly linked to *Arcobacter butzleri*, *A. cryaerophilus* and *A. skirrowii* (Collado & Figueras).

Human Infection can be induced by contact with infected people and animals and by the consumption of contaminated water, vegetables and milk (Nauta *et al.*, 2009; Allain *et al.*, 2014; González *et al.*, 2016; Heredia & García, 2018). Species that belong to the *Campylobacteraceae* family also reside in the gastrointestinal tract of food producing animals such as poultry, cattle, sheep and pigs (Shange *et al.*, 2019). In fact, contaminated undercooked/partially cooked meat and meat products is the main source of infection, literature places a special emphasis on poultry meat and products (González *et al.*, 2016). Poultry species are viewed as the primary reservoir for *Campylobacteraceae* species, implying successful colonisation during the life cycle of poultry whilst still at primary production (farm level).

At primary production, horizontal transmission has been widely reported as successful in transferring *Campylobacter* and *Arcobacter* species from the vectors such as domestic pets, flies, insects, water, migratory birds, farm equipment, transport vehicles, feed, farm workers, rodents and litter to flocks/herd (Stanley & Jones 2003; Kalupahana *et al.* 2018; Prince Milton *et al.*, 2017; Shange *et al.*, 2019). Evidence of this notion has been seen in the high genetic diversity of isolates isolated from farm animals, possibly indicating multiple sources of *Campylobacteraceae* species at primary production (De Smet *et al.* 2011). An onset of an infection through horizontal transmission at primary production can occur when an animal is a few weeks old. Considerable efforts have been made to study the onset of *Campylobacter* and *Arcobacter* species in food-producing animals. Understanding the mechanisms of colonisation and the time point of colonisation could help contribute to possible prevention and control strategies at primary level (at the farm) (Cawthraw & Newell, 2010). However, as it stands, most of the studies that have investigated the onset of species that belong to the *Campylobacteraceae* family are based on broiler chickens.

The ostrich industry can be classified as an intensive livestock production system, particularly as pertaining to the younger birds. Hatchlings can be sold to growers or reared to the age of three to six months before being sold onto producers or, the primary hatchery can be vertically integrated and raise the hatchlings to slaughter age (10-14 months) (Engelbrecht, 2014). Ostrich chicks can be artificially or naturally reared, where the latter includes the rearing of ostrich chicks in groups of 30-50 in small paddocks during the day and providing shelter inside a building that is temperature regulated and ventilated at night. As the ostrich chicks gain the ability to regulate body temperature, they are kept in paddocks during the day and night (Engelbrecht, 2014). Ostrich chicks can also be reared naturally, in groups of 10-15 with foster parents on irrigated Lucerne

(alfalfa (*Medicago sativa*)) pastures (Engelbrecht, 2014). Intensification of the rearing system can create an ideal environment for the development and spreading of diseases, surprisingly little is known about the onset and presence of *Campylobacter* and *Arcobacter* species in ostriches. Therefore, the aim of this study was to investigate the onset of *Campylobacter* spp. and *Arcobacter* spp. in artificially and naturally reared ostrich chicks and subsequently to look into the possible prevalence of *Campylobacter* spp. and *Arcobacter* spp. in slaughter age ostriches.

3.2 Materials and method

3.2.1 Onset of colonisation

3.2.2 Farm practice

At this farm, ostrich chicks were artificially or naturally reared. Artificial rearing required ostrich chicks to be kept in temperature-controlled housing facilities, with artificial lighting, while feed and clean water was supplied *ad libitum*. Frequent human intervention was employed for cleaning and refilling feeding troughs and water containers (Table 3.1). Natural rearing involved the rearing of chicks by a surrogate breeding pair of ostriches in irrigated pastures until ostrich chicks reached the age of ≥12 weeks. Artificially and naturally reared chicks had access to combination of farm produced and commercial feed (Table 3.1).

Table 3.1 Ostrich chick management for artificial and natural rearing system

Artificially - reared ostrich chicks			
Description	**Age range**		
	<4 weeks	**>4 weeks**	**≥12 weeks**
Sleeping area description	Small housing facilities with grids	Larger soil camps	Grazing camps; Lucerne pastures
Type of feed provide	Commercial and farm produced feed	Commercial and farm produced feed	Commercial and farm produced feed
Frequency for cleaning and refilling water and feed	4-6 times a day	4 times a day	Twice a day
Naturally - reared ostrich chicks			
Sleeping area description	Lucerne pastures	Lucerne pastures	Lucerne pastures
Type of feed provide	Commercial feed	Commercial and farm produced feed	Commercial and farm produced feed
Frequency for cleaning and refilling water and feed	4-6 times a day	4 times a day	Twice a day

At this farm, farm management practices that focused on disease control, hygiene practices and rodent control were in place (Table 3.2).

Table 3.2 Ostrich chick farm management and hygiene practices

Parameter	Description
Cleaning and disinfection	Vehicles were cleaned and disinfected.
	Footbaths were present for farm workers
	Walkways were sprayed with disinfectant
	Housing facilities were cleaned and disinfected between batches.
	Faecal matter in housing facilities were raked daily.
	When ostrich chicks slept on grids, grids were cleaned at least weekly
Wildlife	Wild birds such as passerines, doves, starlings, ibises and geese were present
Rodent control	Rodents from neighbouring fields might be present due to spilt or left-over feed. However, this farm tried to keep feed in a sheltered area.
Health status for ostrich chicks	Tetracycline was administered when ostrich chicks were diagnosed with diarrhoea

3.2.3 Sample collection

A total of 24 ostrich chicks were sampled during the year 2018. Sampling commenced in April 2018 to June 2018, which coincided with chick season at which ostrich chicks would be available at this particular farm. Ostrich chicks were reared artificially and naturally with three ostrich chicks being sampled per age group; sampling was done at 2 weeks of age (n = 6), 4 weeks of age (n = 6), 6 weeks of age (n = 6), and at 12 weeks of age (n = 6). At the time of sampling, ostrich chicks showed no signs of disease/sickness. Ostrich chicks were euthanised by cervical dislocation and severance of the spinal cord (ethical clearance number SU-ACUD16-00070). Euthanisation was by a qualified veterinarian who was familiar with the study and was competent at euthanising of ostrich chicks in order to obtain caeca samples from the gastrointestinal tract. Caeca samples were excised from the rest of the gastrointestinal tract by tying off a section of the caeca, then excising a piece of the caeca. Tying off a piece of the caeca was to retain the natural environment of the gastrointestinal tract during sample transportation. Samples were individually packed into labelled sampling bags, kept at 4⁰C and transported to the Food Science department (Stellenbosch University). In general, samples reached the Food Science department for analysis 48 - 72 hours after sampling.

3.2.4 Prevalence of *Campylobacter* and *Arcobacter* spp. at slaughter

Regardless of the rearing processes mentioned above, at 12-16 weeks cohorts of ostrich chicks from the same flocks described above were tagged and moved to a neighbouring ostrich farm in Oudtshoorn, Western Cape. Climatically, this farm could be characterised as cooler and moister. Farm management included providing farm produced feed to ostriches and replacing feed and water only twice a day. At the slaughter age of 10 and 12 months, 30 cloacal swabs were taken from live ostriches at the farm. Once collected, each swab was placed in modified Cary Blair medium.

3.2.5 Cultural isolation of *Campylobacter* and *Arcobacter* species

Campylobacter spp. and *Arcobacter* spp. were non-selectively detected using the Cape Town protocol (Lastovica, 2006). For the Cape Town protocol Tryptose blood agar (TBA) (CM0233 - Oxoid, Basingstoke, United Kingdom) plates enriched with 10% horse blood (Medical Research Council, Delft, South Africa) were used for analysis. Processing of samples using the Cape Town protocol occurred as follows: caeca samples (1g) were placed in peptone water (1:10 w/v) massaged by hand until contents were mixed well. After mixing, the suspension was then incubated at 37ºC for 24 h under micro-aerophilic conditions. After incubation, 200 µl of the enrichment was transferred to TBA plates which contained a sterile cellulose nitrate filter (0.65 µm and 8 µm pore size) (Sartorius Stedim Biotech, Germany) on the surface. The enrichment was transferred in a drop wise motion and the enrichment was left to passively pass through the filter at 37ºC for 15-20 min. Thereafter, the filters were carefully removed. TBA plates were then incubated for 6 days at 37ºC under micro-aerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) achieved using CampyGen sachets (CN00026 - Oxoid, Basingstoke, United Kingdom). Growth on TBA Plates was monitored every 2-days, at each 2-day interval CampyGen sachets were replaced.

Campylobacter spp. was selectively detected using the International Organization for Standardization (ISO) 10272-1:2006 method. Briefly, caeca samples (1g) were inoculated into Bolton broth (CM0983 - Oxoid, Basingstoke, United Kingdom) (1:10 w/v) supplemented with the corresponding supplement (which contained 10 mg of cefoperazone, 10 mg of vancomycin, 10 mg of trimethoprim and 25 mg of cycloheximide (SR0183E - Oxoid, Basingstoke, United Kingdom) and 10% horse blood (Medical Research Council, Delft, South Africa)**.** Sample suspension was massaged by hand, until suspension was mixed well. Sample suspension was then incubated at 37ºC for 4-6 h for the purpose of resuscitation and later incubated at 42ºC for 44-48 h for the purpose of enrichment. Both resuscitation and enrichment occurred under micro-aerophilic conditions achieved by CampyGen sachets (CN00026 - Oxoid, Basingstoke, United Kingdom). After enrichment, a loopful (10 µl) of the enrichment was streaked onto TBA and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (Oxoid, Basingstoke, United Kingdom) supplemented with corresponding supplement (SR0155E - Oxoid, Basingstoke, United Kingdom) which contained cefoperazone (16 mg) and amphotericin B (5 mg)**.** Plates were incubated at 42ºC under micro-aerophilic conditions for 48 h.

Lastly, a portion of the caeca was used for the selective detection of *Arcobacter* spp. Briefly, caeca samples (1g) were inoculated into *Arcobacter* broth (CM0965 - Oxoid, Basingstoke, United Kingdom) (1:10 w/v) supplemented with the corresponding supplement (SR0174E - Oxoid, Basingstoke, United Kingdom)**.** Sample suspensions were massaged by hand, thoroughly mixed and then incubated aerobically at 30ºC for 48 h. After incubation, a loopful (10 µl) of the enrichment was streaked onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (CM0739 - Oxoid, Basingstoke, United Kingdom) supplemented with corresponding supplement (SR01745E - Oxoid, Basingstoke, United Kingdom) which contained cefoperazone (4 mg), teicoplanin (2 mg) and amphotericin B (5 mg)**.** Plates were incubated at 30ºC under aerobic conditions for 48 h (Merga *et al.*, 2013).

Cloacal swabs were placed in tubes containing 3 ml of peptone water and vortexed for one minute. After vortexing a 1:10 dilution (v/v) was made with Bolton broth and *Arcobacter* broth and processed as

indicated above. 200 µl of the left-over sample (1 ml) suspension was incubated and processed according to the Cape Town protocol as previously described for caeca samples.

Suspect *Campylobacter* and *Arcobacter* colonies (four colonies per sample) were streaked onto Columbia blood agar (CBA) (CM0331 - Oxoid, Basingstoke, United Kingdom) enriched with 5% horse blood (Medical Research Council, Delft, South Africa). Typical *Campylobacter* (incubated for 48h at 42°C) and *Arcobacter* (incubated for 48h at 30°C) colonies were tested for genus specific phenotypical and biochemical characteristics such as gram's staining, oxidase, catalase and grown under different atmosphere/temperature conditions. For gram staining, pure colonies grown on CBA were suspended on a glass slide and fixed with heat and stained using a commercially available kit (Sigma-Aldrich, St. Louis, United States of America), stained slides were inspected under a microscope, where a pink stain was considered a positive reaction. For oxidase colonies were spread on commercially available oxidase strips (Merck, Darmstadt, Germany), the appearance of violet or deep blue colour within 10 sec was seen as a positive reaction. Catalase activity was determined through mixing a pure colony with one drop of hydrogen peroxide solution (3%), a rapid (10 sec) formation of bubbles was considered a positive reaction. Lastly, a growth study was conducted, whereby presumptive *Campylobacter* spp. colonies, were streaked on two CBA plates and incubated at 25°C under micro-aerobic atmosphere for 40 h to 48 h and the other plate was incubated at 42°C aerobic conditions for 40 h to 48 h, growth under these conditions was regarded as a negative reaction. Similarly, presumptive *Arcobacter* spp. colonies were streaked on CBA plates and placed under micro-aerophilic atmosphere at 42°C, growth under these conditions was regarded as a negative reaction.

Confirmed colonies were stored in Microbank™ vials (Davies Diagnostics, Johannesburg, South Africa) with beads. Briefly, pure colonies were picked up with a plastic loop and transferred to Microbank™ vials which obtained 25 beads (3 mm) and a cryoprotectant liquid (1 ml) containing 10-15 % glycerol. Microbank™ vials were closed tightly and inverted five times in order to emulsify the microorganism. In order to let the microorganism bind with beads, Microbank™ vials were left to stand for 2 min without disturbance, thereafter the cryoprotectant liquid was aseptically removed and Microbank™ vials with isolates were stored at - 80⁰C until molecular analyses could commence.

3.2.6 Quality control

At each incubation point, control strains: *C. jejuni* subsp. *jejuni* ATCC 29428 (Davies Diagnostics, Johannesburg, South Africa), *C. coli*, *A. butzleri*, *A. skirrowii* and *A. cryaerophilus* were grown and streaked onto the appropriate agar and incubated under the conditions depicted for ISO 10272-1:2006, selective *Arcobacter* spp. method and the Cape Town protocol. Furthermore, a sterility test was conducted on all prepared plates by incubating plates for 24 h at 37°C, any growth on the plates was taken as a sign of contamination, and these plates were then discarded. Control strains were used as positive controls for the molecular confirmation of *Arcobacter* spp. and *Campylobacter* spp. (described below).

3.2.7 Molecular confirmation of *Arcobacter* and *Campylobacter* species

Previously preserved colonies were streaked onto CBA and single colonies were suspended in 300 lysis buffer (10 mM Tris-HCl, 1% Triton X-100, 0.5 % TWEEN 20 and 1 mM EDTA) and then boiled (100⁰C) for 10 min.

After boiling the bacterial suspension was centrifuged at 5500 x g for 2 min. Where after, 250 µl of supernatant were transferred to new Eppendorf tubes with ice cold 99.9% ethanol. Thereafter, the equal parts supernatant and 99.9% ethanol were centrifuged at 13 000 x g for 2 min, the supernatant was discarded, and the pellet was left to dry at room temperature for 1 – 2 h. After drying, 100 µl of 10 mM Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA) was added to the pellet and then mixed by vortexing, at such a point extracted DNA could be used for Polymerase Chain Reaction (PCR) amplification using the primer set depicted in Table 3.3.

Table 3.3 Primer sequences and PCR conditions for the detection of *C. jejuni, C. coli, A. butzleri, A. skirrowii and A. cryaerophilus*

Identification	Sequence (5' to 3')	PCR conditions
Species specific primers (Negahdari *et al.*, 2016)		
C. jejuni	CTATTTTATTTTTGAGTGCTTGTG	one cycle of 2 min at 94ºC, 30 cycles each consisting of 40 sec at 94ºC, 40 sec at 54°C, 5 min at 72°C and a final extension step of 5 min at 72ºC
	GCTTTATTTGCCATTTGTTTTATTA	
C. coli	AATTGAAAATTGCTCCAACTATG	
	TGATTTTATTATTTGTAGCAGCG	
Species specific primers (Soma, 2016)		
A. butzleri	CCTGGACTTGACATAGTAAGAATGA	one cycle of 5 min at 94ºC, 30 cycles each consisting of 30 sec at 94ºC, 1 min at 51ºC, 1 min at 72°C and a final extension step of 10 min at 72ºC
	CGTATTCACCGTAGCATAGC	
A. skirrowii	GGCGATTTACTGGAACACA	
	CGTATTCACCGTAGCATAGC	
A. cryaerophilus	TGCTGGAGCGGATAGAAGTA	
	AACAACCTACGTCCTTCGAC	

PCR mixtures (25 µl) consisted of 10x PCR buffer, 10 mM dNTP's, forward and reverse primers (10 pmol/µl), *Taq* DNA polymerase (1U/µl), sterile distilled water and either 1 µl (for *C. jejuni* and *C. coli*) or 2.5 µl (for *A. butzleri, A. skirrowii* and *A. cryaerophilus*) of extracted DNA. All reagents used for the PCR reaction mixture were purchased from Inqaba Biotec, South Africa. PCR mixtures were subjected to the conditions depicted in Table 3.3. PCR was performed in Bio-Rad T100 Thermal Cycler (Bio-Rad, South Africa). A 1.5% agarose gel stained with EZ vision was used for the gel electrophoresis of PCR products (10 µl). All gels ran for 90 min at 70 V. A 100 bp DNA ladder was used for each run, in order to size the products. A Bio-Rad Gel Doc XR+ System (Bio-Rad, South Africa) and Image Lab Software (version 5.2.1) was used for gel visualisation.

3.3 Statistical analysis

Prevalence for each sampling date (age: 2 weeks, 4 weeks, 6 weeks, 12 weeks, 10 months and 12 months) was calculated by dividing the number of samples from which a *Campylobacter* spp. and *Arcobacter* spp. isolate was obtained by the total number of samples processed. Data was used for the configuration of all tables.

3.4 Results

Campylobacter spp. were only detected at the age of 12 weeks from ostrich chicks reared artificially, whilst no *Campylobacter* spp. was isolated from naturally reared ostriches (Table 3.4). Furthermore, two isolation protocols were used for the recovery for *Campylobacter* spp.; with the ISO 10272-1:2006 method a low overall prevalence of 8% (2/12) was determined, whilst the Cape Town protocol could not successfully recover *Campylobacter* spp.

In contrast to the low recovery seen for *Campylobacter* spp., *Arcobacter* spp. showed an efficient introduction, as it was present from the first sampling age point (2 weeks) for both artificially and naturally reared ostrich chicks (Table 3.5). Two isolation protocols were used to determine the colonisation of *Arcobacter* spp.; both the Cape Town protocol and the selective method succeeded in detecting *Arcobacter* spp. However, the selective method was slightly more efficient, as a 100% (12/12) prevalence was determined for artificially and naturally reared ostrich chicks. whilst the Cape Town protocol only determined a prevalence of 75% (9/12) and 67% (8/12) for artificially and naturally reared ostrich chicks, respectively.

As seen in Tables 3.4 and 3.5, *Campylobacter* spp. and *Arcobacter* spp. were detected in slaughter-aged ostriches at prevalence levels ranging from 57% to 70%. The Cape Town protocol was unsuccessful in detecting *Campylobacter* spp. in cloacal swabs but could detect *Arcobacter* spp.

Table 3.4 Overview of *Campylobacter* spp. isolations from artificially and naturally reared ostrich chick and slaughter-aged ostriches

Age	*Campylobacter* spp. positive samples[#]			
	Cape Town protocol		**ISO 10272-1:2006**	
	Artificial	**Natural**	**Artificial**	**Natural**
2 weeks (n = 3)*	0/3	0/3	0/3	0/3
4 weeks (n = 3)	0/3	0/3	0/3	0/3
6 weeks (n = 3)	0/3	0/3	0/3	0/3
12 weeks (n = 3)	0/3	0/3	2/3	0/3
Overall prevalence (%)	-	-	2/12 (8%)	-
Slaughter age	**Cape Town protocol**		**ISO 10272-1:2006**	
10 months (n = 30)	0/30		17/30	
12 months (n = 30)	0/30		21/30	

*Number of samples tested

[#] Number samples positive/number samples tested

Table 3.5 Overview of *Arcobacter* spp. isolations from artificially and naturally reared ostrich chick and slaughter-aged ostriches

Age	*Arcobacter* spp. positive samples[#]			
	Cape Town protocol		**Selective *Arcobacter* spp. method**	
	Artificial	**Natural**	**Artificial**	**Natural**
2 weeks (n = 3)*	2/3	2/3	3/3	3/3
4 weeks (n = 3)	3/3	1/3	3/3	3/3
6 weeks (n = 3)	1/3	2/3	3/3	3/3
12 weeks (n = 3)	3/3	3/3	3/3	3/3
Overall prevalence (%)	9/12 (75%)	8/12 (67)	12/12 (100%)	12/12 (100%)
Slaughter age	**Cape Town protocol**		**Selective *Arcobacter* spp. method**	
10 months (n = 30)	20/30		NT	
12 months (n = 30)	17/30		21/30	

NT = Not tested

[#] Number samples positive/number samples tested

*Number of samples tested

In Table 3.6 *Campylobacter* spp. and *Arcobacter* spp. isolates were further distinguished as *Campylobacter jejuni* (*C. jejuni*) and *Arcobacter skirrowii* (*A. skirrowii*).

Table 3.6 A summary of *Campylobacter* and *Arcobacter* species as confirmed with PCR

Isolate origin	*C. jejuni*	*C. coli*	*A. butzleri*	*A. cryaerophilus*	*A. skirrowii*
2 weeks	-	-	-	-	P[1]
4 weeks	-	-	-	-	p
6 weeks	-	-	-	-	p
12 weeks	P	-	-	-	p
10 months	P	-	-	-	P
12 months	P	-	-	-	P

[1] Species confirmed through PCR

3.5 Discussion

3.5.1 Onset of *Campylobacter* and *Arcobacter* infection in ostrich chicks

The onset of *Campylobacter* spp. was only seen with the ISO selective method on the 12th week for artificially reared chicks, whilst the non-selective Cape Town protocol could not successfully detect *Campylobacter* spp. (Table 3.4 and 3.6). As seen in the materials and methods, for the purpose of resuscitation (Ling *et al.*, 2011), a 24-hour enrichment period was included in the non-selective protocol but results from this study suggest that the enrichment period was not long enough for the resuscitation of *Campylobacter* spp. *Campylobacter* spp. are known to be extremely sensitive to unfavourable conditions and when stressed, *Campylobacter* cells tend to go into a viable but not culturable state (Humphrey *et al.*, 2007). Typically, an enrichment period will rectify this state and allow for cultivation. However, for this study it would seem that stress experienced during the 48-hour transport period, in an unsuitable environment, could not be rectified by the 24-hour enrichment

period. Nonetheless, detection was achieved with the ISO method, which could be attributed to the longer incubation period (Hayashi *et al.*, 2013) characterised by 4-6 hours for resuscitation and 48-hour for enrichment (ISO, 2006). It is also noteworthy that subsampling of caeca samples and/or potential temperature fluctuations during the transportation of samples could have impacted results.

With regards to the colonisation of ostrich chicks with *Campylobacter* spp., studies focusing on the colonisation of *Campylobacter* spp. in poultry chicks show that an asymptomatic infection is unlikely to occur before 2-3 weeks of age; as seen by Berndtson *et al* (1996) who tested over 24 chicken flocks and did not find evidence of colonised chicks that were one week of age. Similarly, Niell *et al.* (1984) tested 12 broiler chicken flocks and could not find evidence of colonisation in chicks that were one and two weeks of age. Furthermore, Rogol *et al.* (1986) and Saleha (2004) tested broiler chicks within the age range of 1 day to 8 weeks and 1 day to 3 weeks, respectively, and found that 1 day old broiler chicks examined were negative for *Campylobacter* spp. It is thought that a natural infection undergoes a lag phase for the first 2-3 weeks of a chick's life. The lag phase can be defined as the period preceding an infection and has been mainly attributed to maternal antibodies from infected parent stock, inhibiting colonisation (Allen *et al.*, 2011a) and also to the natural intestinal flora of young chicks inhibiting colonisation (Van der Wielen *et al.*, 2000). However, after the lag phase; as age transcends the 2-3 week mark, a colonisation can be expected as seen in broiler chickens and turkeys (Berndtson *et al.*, 1996; Saleha, 2004; El-Adawy *et al.*, 2012). Therefore, an immediate colonisation after the lag phase was expected in this study. However, for this study an extended lag phase was seen, as colonisation only occurred at the age of 12 weeks and only for the artificially reared ostrich chicks. Similarly, other studies have reported an extension of the lag phase, for instance Smith *et al.* (2004) reported on two turkey flocks that did not show any colonisation for 11 weeks. Similarly, Lindblom *et al.* (1986), also reported a longer than normal lag phase in chickens, for this case *Campylobacter* spp. was only seen on the 5th week. The extended lag phase or delay in colonisation was attributed to the notion that *Campylobacter* spp. were not present on the farm until the 5th week. The same principle could be applied to this study. For the farm sampled it was considered part of normal farm practice to move ostrich chicks to grazing camps once they reach the age of 12 weeks (Table 3.1). Therefore, there is a possibility that *Campylobacter* spp. was not present in the housing facilities, but present on the grazing camps. This would also indicate that the standard cleaning and sterilisation program applied in the whole facility between ostrich chick batches is efficient. During this time, it is feasible to assume that the ostrich chicks had more exposure to the environment and possible sources of *Campylobacter* spp. (Vandeplas *et al.*, 2010). Additionally, even though the sample size for this study was small, it was possible to exhibit an extended lag phase, and identify the 12th week of life, as a possible point of colonisation for artificially reared chicks. However, in a study of *Campylobacter* infections in four poultry species (broiler chickens, ducks, Muscovy ducks and turkeys), a distinct point of introduction/ colonisation could not be pin pointed (Weber *et al.* 2014).

Another possible reason for the delay in colonisation could be attributed to the administered tetracycline to treat enteritis. For this particular farm it was considered normal farm practice to treat ostrich chicks diagnosed with diarrhoea with tetracycline. During the rearing process (from the 2nd to the 12 week of life) some of the ostrich chicks suffered from enteritis. Tetracycline is an antibiotic used to treat a broad number

of infections (Simango, 2013); the use of tetracycline could have doused an early onset of the infection of *Campylobacter* spp. At least 91% of *Campylobacter* spp. isolates from chickens from South Africa were found susceptible to tetracycline (Simango, 2013). The use of antibiotics during the early days of a chick's life could change the natural flora of the digestive system and subsequently prevent the initial colonisation of *Campylobacter* spp. in the digestive system (Scuphamn *et al.,* 2010; Ansari-Lari *et al.*, 2011). It is therefore, reasonable to postulate that the onset of *Campylobacter* spp. could be inhibited by the use of tetracycline. In saying that, other studies have also investigated *Arcobacter* in relation to antibiotic resistance, and showed that some *Arcobacter* spp. strains have the potential to resist tetracycline (Adesiji *et al.*, 2011). This notion could explain why the asymptomatic *Arcobacter* spp. infection flourished and *Campylobacter* spp. infection was inhibited.

A persistent introduction of *Arcobacter* spp. was determined from 2 weeks (beginning point of sampling), until the 12th week. Evidence of *Arcobacter* spp. has been proven during the slaughter of poultry species (Van Driessche & Houf, 2007). However, previously there had been discrepancies within research as to whether *Arcobacter* spp. are commensal microorganisms within the poultry species' gastrointestinal tract, so much so that *Arcobacter* spp.'s presence in poultry carcasses has been attributed to post slaughter contamination. However, since then studies have shown that *Arcobacter* spp. indeed inhabit the gastrointestinal tract of poultry (Ho *et al.*, 2006; Atabay *et al.*, 2006) and have shown evidence of *Arcobacter* spp. during the shedding of faecal matter by poultry species such as chicken, ducks, turkeys and domestic geese (Collado & Figueras, 2011). However, unlike *Campylobacter* spp., *Arcobacter* spp. colonisation has not been extensively investigated; in fact, studies are lacking. This has been attributed to the use of isolation methods that only promote the growth of thermophilic *Campylobacter* species (Van Driessche & Houf, 2007). Despite this reality, Wesley and Baetz (1996) investigated an artificial and natural *Arcobacter* spp. infection in chickens and found that older chickens were infected and found no evidence of an infection in younger chickens. Similarly, Adesiji *et al.* (2011) isolated *Arcobacter* spp. only from older birds, whilst an infection was not established for younger birds. It is known that *Arcobacter* spp. colonisation of the avian gastrointestinal system varies considerably. Therefore, findings from this study might not agree with results expressed previously, as a constant and persistent natural infection (prevalence of 100%) was shown in ostrich chicks from 2 weeks of age (the first sampling point) until 12 weeks of age (Table 3.5). A low detection rate for *Arcobacter* spp. was reported in poultry chickens from Nigeria (Adesiji *et al.*, 2011), these findings suggested that chickens from Nigeria could be a minor source for *Arcobacter* spp. However, for this study, the consistent detection of *Arcobacter* spp. could warrant the opposite where ostriches could potentially be a major source of *Arcobacter* spp.

Currently, it is unknown how environmental factors aid *Arcobacter* spp. in the colonisation of poultry species (Wesley & Baetz, 1996). However, the persistent detection of *Arcobacter* in artificially and naturally reared ostrich chicks seen for this study, suggests a consistent and a possibly shared source of *Arcobacter* spp. at the ostrich chick farm. Other studies have postulated that water sources/ water troughs might play a significant role in aiding infection (Giacometti *et al.*, 2015). In fact, *Arcobacter* spp. are well known for their ability to survive and replicate in water (Banting & Figueras, 2017). However, in order to substantiate this

claim, an investigation into possible sources of *Arcobacter* spp. in ostrich chick farms would need to be conducted.

3.5.2 Prevalence of *Campylobacter* spp. and *Arcobacter* spp. in slaughter-aged ostriches

With respect to the whole project, this part of the study was in fact the first look into a possible prevalence of *Campylobacter* and *Arcobacter* spp. in live slaughter-aged ostriches, reared in South Africa. It should be noted that cohorts that belonged to same batch as the sacrificed ostrich chicks (discussed above), were traced to one farm in the Oudtshoorn area. With this approach it was known that the sampled flock, once comprised of chicks infected with *Campylobacter* and *Arcobacter* spp. For this part of the study, *Campylobacter* spp. and *Arcobacter* spp. infection levels varied from 56 to 70% (Tables 3.4 and 3.5). The presence of *Campylobacter* in slaughter-aged ostriches, is in agreement with Oyarzabal *et al.* (1995), Cuomo *et al.* (2007), Ling *et al.* (2011) and Prince Milton *et al.* (2017). The prevalence in this investigation were higher than the prevalence levels of 40%, 28%, 2% and 6% reported by Cuomo *et al.* (2011), Oyarzabal *et al.* (1995), Ling *et al.* (2011) and Prince Milton *et al.* (2017), respectively. Variation between studies is not uncommon and can be attributed to the varying age of animals, sampling season and methods used for sampling and detection (Andrzejewska *et al.*, 2013). Additionally, comparisons between studies are challenging as previous research sampled ostriches randomly to determine a prevalence, whilst for this study, a flock that obtained the cohorts of infected ostriches were sampled.

Regarding *Arcobacter* spp., evidence from other studies that have isolated *Arcobacter* spp. from ostriches could not be found, therefore, an incidence rate ranging from 56-70% potentially provides evidence that ostriches could be a reservoir for *Arcobacter* species, similar to other poultry species such as chickens, turkeys, ducks and geese (Hassan, 2017; ÇelÌk *et al.*, 2018).

3.6 Conclusion

The present study indicates that the onset of *Campylobacter* spp. colonisation could be influenced by farm practices, as some practices such as moving ostrich chicks to grazing camps can allow for an opportunity to introduce *Campylobacter* spp. to ostrich chicks. Furthermore, this study showed a persistent presence of *Arcobacter* spp. from 2 weeks on-wards, despite the rearing practices used. These findings suggest that ostrich chick farms could be important sources of *Arcobacter* spp. However, a risk assessment to determine potential sources of *Arcobacter* spp. is required.

This study also indicated that in a typical ostrich farm with slaughter-aged ostriches, *Campylobacter* spp. and *Arcobacter* spp. could prevail until the age of slaughter, once it has entered a flock. The high prevalence could be an indication that once ostriches are infected, they can remain infected until the age of slaughter and can subsequently infect other ostriches within the same flock resulting in higher prevalence levels. To our knowledge this is one of the few studies, if not the only study to isolate *Arcobacter* spp. in ostrich chicks and in live slaughter-aged ostriches.

As expressed in the discussion, this study was limited in sample size and only one farm was used to prove a prevalence at slaughter age/primary level. Therefore, a recommendation to be taken further and

implemented would be to sample more farms so as to obtain a larger sample size in order to conclusively determine the incidence rate of *Campylobacter* spp. and *Arcobacter* spp. in live ostriches from South Africa.

3.7 References

Adesiji, Y., Alli, O., Adekanle, M. & Jolayemi, J. (2011). Prevalence of *Arcobacter*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone Journal of Biomedical Research*, **3**, 8–12.

Allain, V., Chemaly, M., Laisney, M.J., Rouxel, S., Quesne, S. & Le Bouquin, S. (2014). Prevalence of and risk factors for *Campylobacter* colonisation in broiler flocks at the end of the rearing period in France. *British Poultry Science*, **55**, 452–459.

Allen, V.M., Ridley, A.M., Harris, J.A., Newell, D.G. & Powell, L. (2011). Influence of production system on the rate of onset of *Campylobacter* colonization in chicken flocks reared extensively in the United Kingdom. *British Poultry Science*, **52**, 30–39.

Altekruse, S.F. & Tollefson, L.K. (2003). Human campylobacteriosis: a challenge for the veterinary profession. *Journal of the American Veterinary Medical Association*, **223**, 445–452.

Andrzejewska, M., Szczepańska, B., Klawe, J.J., Śpica, D. & Chudzińska, M. (2013). Prevalence of *Campylobacter jejuni* and *Campylobacter coli* species in cats and dogs from Bydgoszcz (Poland) region. *Polish Journal of Veterinary Sciences*, **16**, 115–120.

Ansari-Lari, M., Hosseinzadeh, S., Shekarforoush, S.S., Abdollahi, M. & Berizi, E. (2011). Prevalence and risk factors associated with *Campylobacter* infections in broiler flocks in Shiraz, southern Iran. *International Journal of Food Microbiology*, **144**, 475–479.

Atabay, H.I., Wainø, M. & Madsen, M. (2006). Detection and diversity of various *Arcobacter* species in Danish poultry. *International Journal of Food Microbiology*, **109**, 139–145.

Banting, G. & Figueras, S. M. J. (2017). Global Water Pathogen Project. [Internet document]. URL http://www.waterpathogens.org/book/*Arcobacter* 08/08/2018.

Barboza, K., Angulo, I., Zumbado, L., Redondo-solano, M. & Castro, E. (2017). Isolation and Identification of *Arcobacter* Species from Costa Rican Poultry Production and Retail Sources. *Journal of Food Protection*, **80**, 779–782.

Berndtson, E., Danielsson-Tham, M.L. & Engvall, A. (1996). *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. *International Journal of Food Microbiology*, **32**, 35–47.

Cawthraw, S.A. & Newell, D.G. (2010). Investigation of the presence and protective effects of maternal antibodies against *Campylobacter jejuni* in chickens. *Avian Diseases Digest*, **5**, e30–e31.

Çelİk, E., Sağlam, A.G., Çelebİ, Ö. & Otlu, S. (2018). Isolation of *Arcobacter* spp . from domestic ducks and geese and identification of the recovered isolates by using molecular method. *Turkish Journal of Veterinary and Animal Sciences*, **42**, 1–6.

Collado, L. & Figueras, M.J. (2011). Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clinical Microbiology Reviews*, **24**, 174–192.

Cuomo, A., Dipineto, L., Santaniello, A., Matteoli, G., Sarli, T., Vecchia, D. Della, Fioretti, A. & Menna, L.F. (2007). Detection of thermotolerant *Campylobacter* in ostriches (*Struthio camelus*) in Italy. *Veterinary Journal*, **174**, 439–441.

De Smet, S., Zutter, L. De, Debruyne, L., Vangroenweghe, F., Vandamme, P. & Houf, K. (2011). *Arcobacter* population dynamics in pigs on farrow-to-finish farms. *Applied and Environmental Microbiology*, **77**, 1732–1738.

ECDC & EFSA (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA Journal*, **13**, 1–231.

El-Adawy, H., Hotzel, H., Tomaso, H., Neubauer, H. & Hafez, H.M. (2012). Elucidation of colonization time and prevalence of thermophilic *Campylobacter* species during turkey rearing using multiplex polymerase chain reaction. *Poultry Science*, **91**, 454–459.

Engelbrecht, S. (2014). Artificial rearing of chicks. In: Ostrich manual (edited by P. Jorgensen) Pp 63-68. Western Cape: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Giacometti, F., Lucchi, A., Francesco, A. Di, Delogu, M., Grilli, E., Guarniero, I., Stancampiano, L., Manfreda, G., Merialdi, G. & Serraino, A. (2015). *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii* circulation in a dairy farm and sources of milk contamination. *Applied and Environmental Microbiology*, **81**, 5055–5063.

González, M., Mikkelä, A., Tuominen, P., Ranta, J., Hakkinen, M., Hänninen, M. & Llarena, A. (2016). Risk assessment of *Campylobacter* spp. in Finland. [Internet document]. URL https://www.evira.fi/globalassets/tietoa-evirasta/julkaisut/julkaisusarjat/elintarvikkeet/risk-assessment-of-*Campylobacter*-spp.pdf. 08/02/2017.

Hassan, A.K. (2017). Detection and Identification of *Arcobacter* species in poultry in Assiut Governorate, Upper Egypt. *Journal of Advanced Veterinary Research*, **7**, 53–58.

Hayashi, M., Kubota-Hayashi, S., Natori, T., Mizuno, T., Miyata, M., Yoshida, S., Zhang, J., Kawamoto, K., Ohkusu, K., Makino, S. & Ezaki, T. (2013). Use of blood-free enrichment broth in the development of a rapid protocol to detect *Campylobacter* in twenty-five grams of chicken meat. *International Journal of Food Microbiology*, **163**, 41–46.

Heredia, N. & García, S. (2018). Animals as sources of food-borne pathogens: A review. Animal *Nutrition Journal*, **2018**, 4–9.

Ho, H.T.K., Lipman, L.J.A. & Gaastra, W. (2006). *Arcobacter*, what is known and unknown about a potential foodborne zoonotic agent! *Veterinary Microbiology*, **115**, 1–13.

Humphrey, T., O'Brien, S. & Madsen, M. (2007). *Campylobacters* as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, **117**, 237–257.

ISO 10272:1 (2006). Microbiology of food and animal feeding stuffs horizontal method for detection and enumeration of *Campylobacter* spp. URL https://www.iso.org/standard/37091.html. 03/01/2017.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, **28**, 687–720.

Kalupahana, R.S., Kottawatta, S.A. & Somarathne, S. (2018). Weather correlates of *Campylobacter* prevalence in broilers at slaughter under tropical conditions in Sri Lanka. *Epidemiology and infection*, **56**, 1–8.

Keener, K.M., Bashor, M.P., Curtis, P.A., Sheldon, B.W. & Kathariou, S. (2004). Comprehensive review of *Campylobacter* and poultry processing. *Reviews in Food Science and Food Safety*, **3**, 105–116.

Lastovica, A.J. (2006). Emerging *Campylobacter* spp.: The tip of the iceberg. *Clinical Microbiology Newsletter*, **28**, 49–56.

Lastovica, A.J., On, L.W. & Zhang, L. (2014). The Family *Campylobacteraceae*. In: *The prokaryotes-Deltaproteobacteria and Epsilonproteobacteria* (edited by E. Rosenberg *et al.*). Pp. 308 – 326. Berlin: Springer-Verlag.

Lindblom, G.B., Sjögren, E. & Kaijser, B. (1986). Natural *Campylobacter* colonization in chickens raised under different environmental conditions. *Journal of Hygiene*, **96**, 385–391.

Ling, Y.E., Aziz, S.A. & Abu, J. (2011). Occurrence of *Campylobacter* and *Salmonella* spp. in Ostrich. *J. Vet. Malaysia*, **24**, 6–8.

Lund, M., Nordentoft, S., Pedersen, K. & Madsen, M. (2004). Detection of *Campylobacter* spp. in chicken fecal samples by real-time PCR. *Journal of Clinical Microbiology*, **42**, 5125–5132.

Merga, J.Y., Williams, N.J., Miller, W.G., Leatherbarrow, A.J.H., Bennett, M., Hall, N., Ashelford, K.E. & Winstanley, C. (2013). Exploring the diversity of *Arcobacter butzleri* from cattle in the UK using MLST and whole genome sequencing. *PLoS ONE*, **8**, 1–12.

Muller, C.J.C (2014). The housing of ostrich chicks. In: Ostrich manual (edited by P. Jorgensen). Pp 69-74. Western Cape: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., Logt, P. van der, Fazil, A., Christensen, B., Katsma, E., Borck, B. & Havelaar, A. (2009). A comparison of risk assessments on *Campylobacter* in broiler meat. *International Journal of Food Microbiology*, **129**, 107–123.

Negahdari, B., Shirazi, M., Malekshahi, Z.V., Kadkhodazadeh, M., Hajikhani, S. & Rahmati, M. (2016). Identification of *Campylobacter Jejuni* and *Campylobacter coli* from diarrheic samples using PCR. *International Journal of Health Studies*, **2**, 1–3.

Neill, S.D., Campbell, J.N. & Greene, J.A. (1984). *Campylobacter* species in broiler chickens. *Avian Pathology*, **13**, 777–785.

Oyarzabal, O.A., Donald, E.C. & Hoerrb, F.J. (1995). Incidence of *Campylobacters* in the Intestine of avian Species in Alabama. *Avian Diseases*, **39**, 147–151.

Percivalle, E., Monzillo, V., Pauletto, A., Marone, P. & Imberti, R. (2016). Colistin inhibits *E. coli* O157:H7 Shiga-like toxin release, binds endotoxins and protects Vero cells. *New Microbiologica*, **39**, 119–123.

Prince Milton, A.A., Agarwal, R.K., Priya, G.B., Saminathan, M., Aravind, M., Reddy, A., Athira, C.K., Anjay, Ramees, T.P., Dhama, K., Sharma, A.K. & Kumar, A. (2017). Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in captive wildlife species in India. *Iranian Journal of Veterinary Research*, **18**, 177–182.

Prince Milton, A.A., Agarwal, R.K., Priya, G.B., Saminathan, M., Aravind, M., Reddy, A., Athira, C.K., Anjay, Ramees, T.P., Dhama, K., Sharma, A.K. & Kumar, A. (2017). Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in captive wildlife species in India. *Iranian Journal of Veterinary Research*, **18**, 177–182.

Robert, E., Levine, M.M., Lou, M., Hughes, T.P. & Martin, J. (1988). Experimental *Campylobacter jejuni* Infection in humans. *The Journal of Infectious Diseases*, **157**, 472–479.

Rogol, M., Kass, N., Borochovich, E. & Marantz, B. (1986). Incidence of *Campylobacter* spp. In broiler flocks monitored from hatching to slaughter. *Avian Pathology*, **15**, 83–92.

Saleha, A.A. (2004). Epidemiological study on the colonization of chickens with *Campylobacter* in broiler farms in Malaysia: Possible risk and management factors. *International Journal of Poultry Science*, **3**, 129–134.

Sasi Jyothsna, T.S., Rahul, K., Ramaprasad, E.V.V., Sasikala, C. & Ramana, C. V. (2013). *Arcobacter anaerophilus* sp. nov., isolated from an estuarine sediment and emended description of the genus *Arcobacter*. *International Journal of Systematic and Evolutionary Microbiology*, **63**, 4619–4625.

Scupham, A.J., Jones, J.A., Rettedal, E.A. & Weber, T.E. (2010). Antibiotic manipulation of intestinal microbiota to identify microbes associated with *Campylobacter jejuni* exclusion in poultry. *Applied and Environmental Microbiology*, **76**, 8026–8032.

Shange, N., Gouws, P. & Hoffman, L.C. (2019). *Campylobacter* and *Arcobacter* species in food - producing animals: prevalence at primary production and during slaughter. *World Journal of Microbiology and Biotechnology*, **35**, 146.

Simango, C. (2013). Antimicrobial susceptibility of *Campylobacter* species. *South African Journal of Epidemiology and Infection*, **28**, 139–142.

Smith, K., Reimers, N., Barnes, H.J., Lee, B.C., Siletzky, R. & Kathariou, S. (2004). *Campylobacter* colonization of sibling turkey flocks reared under different management conditions. *Journal of food protection*, **67**, 1463–8.

Stanley, K. & Jones, K. (2003). Cattle and sheep farms as reservoirs of *Campylobacter*. *Journal of applied microbiology*, **94**, 104S–113S.

Soma, S.M. (2016). *Studies on Arcobacter species of animal and human origin.* MVSc Thesis. Sri Venkateswara Veterinary University, India

Van der Wielen, P., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B. & Knapen, F. (2000). Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Applied and Environmental Microbiology*, **66**, 2536–2540.

Van Driessche, E. & Houf, K. (2007). Discrepancy between the occurrence of *Arcobacter* in chickens and broiler carcass contamination. *Poultry Science*, **86**, 744–751.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N., Zissis, G. & Butzler, J. (2004). *Arcobacter* species in humans. *Emerging Infectious Diseases*, **10**, 1863-1867.

Vandeplas, S., Dauphin, R.D. & Palm, R. (2010). Prevalence and sources of *Campylobacter* spp. contamination in free-range broiler production in the southern part of Belgium. *Biotechnol. Agron. Soc. Environ.*, **14**, 279–288.

Weber, R., Auerbach, M., Jung, A. & Glünder, G. (2014). *Campylobacter* infections in four poultry species in respect of frequency, onset of infection and seasonality. *Berliner und Münchener tier ärztliche Wochenschrift*, **127**, 257–266.

Wesley, I. V & Baetz, A.L. (1996). Natural and experimental infections of *Arcobacter* in poultry. *Poultry Science*, **78**, *536*–545.

CHAPTER 4

The prevalence of *Campylobacter* and *Arcobacter* species in ostriches from Oudtshoorn, South Africa[†]

Abstract

A total of 836 cloacal swabs were obtained from live ostriches reared on 30 different farms situated in South Africa (Oudtshoorn) during the period of June 2018 to July 2019 in order to determine prevalence of *Campylobacter* and *Arcobacter* species. PCR (n = 168 pooled cloacal swabs), the Cape Town protocol (n = 836 cloacal swabs), ISO 10272-1:2006 (n = 836 cloacal swabs) and a selective *Arcobacter* spp. method (n = 415 cloacal swabs) were used for detection. PCR determined an average prevalence of 24.63% for species belonging to the *Campylobacteraceae* family. The ISO 10272-1:2006 method determined a *Campylobacter* spp. prevalence level of 16.83%, whilst the Cape Town protocol could not detect *Campylobacter* spp. For *Arcobacter* spp. a prevalence of 18.80% and 39.14% was determined with the Cape Town protocol and the selective *Arcobacter* spp. method, respectively. Prevalence levels determined by the ISO 10272-1:2006 and the selective *Arcobacter* spp. method were used to evaluate the effect of risk factors. Results showed that prevalence levels could be influenced by season, the source of water and the presence of wild water birds. Higher prevalence levels for *Campylobacter* spp. (23.38%) and *Arcobacter* spp. (68%) were detected in ostriches sampled during spring and autumn, respectively. Higher prevalence levels for *Campylobacter* spp. (25.23%) and *Arcobacter* spp. (44.50%) were detected in ostriches reared on farms that made use of borehole water. Higher prevalence levels for *Arcobacter* spp. (44.38%) were seen in ostriches reared on farms with wild water birds. This research shows that ostriches from South Africa can be considered as potential carriers of species belonging to the *Campylobacteraceae* family.

4.1 Introduction

South Africa is the undisputed world leader as pertaining to the ostrich industry and is renowned for exporting ostrich feathers, leather and meat (Jorgensen, 2014). The consistent demand for ostrich meat has been attributed to a change in consumer dietary preference (Alonso-Calleja. *et al.*, 2004), as ostrich meat can provide the health-conscious consumer with an alternative protein that is lean, low in cholesterol, low in lipid content, high in protein and n-3 polyunsaturated fatty acids (PUFA) contents (Hoffman & Fisher, 2001; Hoffman & Mellet, 2003; Girolami *et al.*, 2003).

Within the ostrich industry (ostrich farmers and ostrich meat producers) there is a lack of awareness towards pathogenic species that belong to the *Campylobacteraceae* family, which encompasses both the *Campylobacter* and *Arcobacter* genera (Lastovica, On & Zhang, 2014). In part, this could be attributed to the fact that regulatory specification for the governing of species that belong to the *Campylobacteraceae* family is

not included within the recently drafted Veterinary Procedural Notices (VPN) 52/2018, which depicts the Standard Operating Procedure (SOP) for microbiological monitoring for meat. *Campylobacter jejun*i (*C. jejuni*) and *Campylobacter coli* (*C. coli*) are recognised as potential microbiological hazards but due to the lack of available data for ostriches, sampling specification have not been set. Additionally, at primary production, species that belong to the *Campylobacteraceae* family are not listed as one of the microorganisms that could potentially cause diseases in ostriches (Olivier, 2014).

Worldwide interest towards species that belong to the *Campylobacteraceae* family has grown possibly due to the implication of *C. jejuni* and *C. coli* in the cause of most of the gastrointestinal infections in humans (Heredia & García, 2018). Additionally, *Campylobacter* spp. has been linked to the cause of more severe illnesses such as Guillain Barré syndrome (Coker *et al*., 2002). In 2010, an overall disease burden of 7.5 million disability adjusted life years (DALY) was attributed to *Campylobacter* related illnesses; this figure was determined by the number of years lost due to ill health, disability and early death. This estimation surpassed the figures reported for *Shigella* and enterotoxigenic *Escherichia coli* (ETEC) (Platts-Mills & Kosek, 2014). Additionally, diarrhoea cases caused by *Campylobacter* spp. have been reported to occur more frequently when compared to more well-known microorganisms such as *Salmonella* (Acheson & Allos, 2001). In 2010, campylobacteriosis was reported to be the sixth most important global burden contributor; C*ampylobacter* species were reported as the most important pathogenic hazard in high-income countries and the second most important microbiological hazard in the European Union (EU) and western Pacific regions by The Foodborne Disease Burden Epidemiology Reference Group (FERG) (Hald *et al.*, 2016). The importance of *Campylobacter* species has also been emphasised by the increase in campylobacteriosis cases reported in developed countries such as Australia, Europe, North and South America (Kaakoush *et al.*, 2015; Heredia & García, 2018) with incidence rates being reported to be 112 (Kaakoush *et al.,* 2015), ≥197 (European Centre for Disease Prevention and Control (ECDC) & European Food Safety Authority (EFSA), 2015), ≥16.18 (Geissler *et al*., 2017) per a population size of 100 000, respectively. The impact of *Campylobacter* spp. is not apparent in all parts of the world, however the most recent report by the ECDC and EFSA, made use of data from 26 EU countries and two European Economic Area (EEA) countries, reported campylobacteriosis as the leading zoonosis, with 246 307 confirmed cases in 2016 (ECDC & EFSA, 2017).

Attention gained by the *Campylobacteraceae* family is also attributed to *Arcobacter* species. *Arcobacter* species are *Campylobacter*-like species that are able to grow under low temperatures and are aerotolerant (Banting & Figueras, 2017). *Arcobacter butzleri* has been deemed an 'emerging' pathogen world-wide and has been classified by the International Commission on Microbiological Specification for Food (ICMSF) as a hazard to human health (Vandenberg *et al*., 2004). Additionally, *A. butzleri* is the fourth most commonly isolated *Campylobacter*-like organism in Belgium and France (Vandenberg *et al*., 2004) and the third most isolated microorganism in South Africa (Samie *et al*., 2007). The potential of *A. butzleri* as a human pathogen is closely followed by *A. cryaerophilus*, *A. skirrowii*, *A. thereius and A. trophiarum* (Banting & Figueras, 2017). Similar to *Campylobacter* spp., *Arcobacter* spp. has been found to be the cause of gastroenteritis in humans and has been linked to the cause of more severe illnesses such as bacteraemia and septicaemia in humans (Banting & Figueras, 2017; Collado *et al*., 2013).

Transmission of *Campylobacter* spp. and *Arcobacter* spp. to humans has been linked to various vectors such as contact with infected animals and humans, as well as contaminated water and raw milk. Additionally, meat and meat products have been reported to be a major source of infection in humans, with a special emphasis being placed on poultry meat and meat products (Evers *et al.,* 2008; Elmali & Can, 2016; Skarp *et al.,* 2016). Furthermore, poultry are deemed the primary reservoir due to the more frequent detection of these organisms in poultry species. According to the South African VPN 04/2012-01 (Revision 6.0) poultry pertains to all domesticated bird species including pigeons, ducks, geese, fowls, turkeys, Muscovy ducks, and domesticated ostriches. As reviewed by Shange *et al.* (2019) prevalence levels of 11-100% for *Campylobacter* spp. and 6-44% for *Arcobacter* spp. have been reported for poultry species such as broiler chickens, geese, ducks and pheasants. Additionally, ostriches from Alabama, Italy, Malaysia and India have been reported to carry *Campylobacter* spp. at prevalence levels ranging from 1.6 to 40% (Ling *et al.*, 2011; Cuomo *et al.*, 2007; Oyarzabal *et al.*, 1995; Prince Milton *et al.*, 2017).

Food-producing animals tend to obtain an asymptomatic infection at primary production (Shange *et al.*, 2019). Even though control strategies are not fully explored, it is speculated that implementation of biosecurity measures at primary production could potentially prevent and/or reduce prevalence levels in food-producing animals. At primary production, the introduction of species that belong to the *Campylobacteraceae* family to farm animals has been linked to the fact that these species are ubiquitous in the environment and can be found in contaminated water sources and soil (Giacometti *et al.* 2015). Furthermore, as reviewed by Shange *et al.* (2019) through horizontal transmission, species that belong to the *Campylobacteraceae* family can be transferred to flocks and herds through domestic pets, flies, insects, farm equipment, farm workers, transport vehicles, litter, pests, rodents and wild migratory birds. For instance, Van Driessche *et al.* (2004; 2005) found that farm workers could act as mechanical vectors, as the same *Arcobacter* strains were isolated from non-adjacent pig pens. Additionally, *Campylobacter* strains found in migratory birds have also been isolated from free-range animals (Colles *et al.* 2008). Furthermore, for poultry the presence of other farm animals (multi-species farming) can increase the likelihood of *Campylobacter* spp. positive flocks (Umar *et al.* 2016).

It has been indicated that an intensive farming practice can possibly perpetuate infections in food-producing animals (Tully & Shane, 1996). This has been attributed to factors such as population densities and possible contamination of water and feed under these farming practices; which ultimately can strain biosecurity measures and can concurrently, increase the risk and spread of infections within flocks/herds (Newell & Fearnley, 2003; Stanley & Jones, 2003). Furthermore, research pertaining to free range farming of food-producing animals shows that the likelihood of colonisation can be increased due to frequent exposure to environmental sources of *Campylobacter* spp. and *Arcobacter* spp. (Newell *et al.*, 2011). For instance, during the study of free-range broiler flocks from Belgium*, Campylobacter* spp. prevalence level of 33.3% to 100% was noted (Vandeplas *et al.*, 2010).

Ostrich rearing is based on three primary productions systems, namely extensive, semi-intensive and intensive (Shanawany & Dingle, 1999). In South Africa, it is estimated that 80% of ostriches in the Klein Karoo region are destined for slaughter, these ostriches are typically reared from three months of age, in an intensive feedlot system while a smaller percentage are raised on pastures (Brand, 2006). The intensive feedlot

system can be characterised by placement of ostriches in small areas of land that have been subdivided into small paddocks (1-2 hectares), with a population density of at least 50 ostriches per 0.5 hectare. In such an intensive feedlot system, there is no grazing and ostriches are fed grower rations *ad libitum* (Brand, 2006). Historically, ostriches came from dry arid environments characterised by low microbial counts and low population densities. However, the intensification of the rearing system for commercial purposes potentially allows for more frequent exposure to harmful organisms, which in turn can affect profitability (Olivier, 2014). This notion in relation to species that belong to the *Campylobacteraceae* family has not been explored. Therefore, this study aims to determine prevalence levels of both *Campylobacter* and *Arcobacter* species in live ostriches reared on South African farms (Oudtshoorn region).

4.2 Materials and method

4.2.1 Sample collection

Without interference to the management system, cloacal swabs were collected from ostriches during the routine testing for avian influenza for reasons linked to slaughter, 6-month survey and outbreak responses. From 30 farms, 836 cloacal swabs were collected from ostriches reared in the Oudtshoorn area (Fig. 4.1) between June 2018 and July 2019 (Table 4.1). All farms used for the study were registered ostrich farms as defined by the VPN 04/2012. From each ostrich farm at the time of sampling, information pertaining to farm characteristics such as multi-species farming, hygiene practices, water sources, presence of pests and population density were collected.

Figure 4.1 A map of the Western Cape, in which the sampling area; Oudtshoorn is shown

Table 4.1 A list of farms sampled for the detection of *Campylobacter* spp. and *Arcobacter* spp.

Farm number (farm code)	Sample date	Number of samples (pooled samples for PCR)
1 (A)	June 2018	30 (6)
2 (B)	July 2018	30 (6)
3 (C)	July 2018	30 (6)
4 (D)	July 2018	25 (5)
5 (E)	July 2018	22 (5)
6 (F)	September 2018	30 (6)
7 (G)	September 2018	2 (1)
8 (H)	September 2018	14 (3)
9 (I)	September 2018	30 (6)
10 (J)	September 2018	30 (6)
11 (K)	September 2018	30 (6)
12 (L)	September 2018	30 (6)
13 (M)	September 2018	30 (6)
14 (N)	November 2018	30 (6)
15 (O)	November 2018	30 (6)
16 (P)	November 2018	30 (6)
17 (Q)	January 2019	30 (6)
18 (R)	January 2019	30 (6)
19 (S)	January 2019	30 (6)
20 (T)	January 2019	30 (6)
21 (U)	January 2019	30 (6)
22 (V)	February 2019	30 (6)
23 (W)	February 2019	30 (6)
24 (X)	February 2019	30 (6)
25 (Y)	February 2019	30 (6)
26 (Z)	May 2019	30 (6)
27 (A_1)	May 2019	30 (6)
28 (B_1)	July 2019	30 (6)
29 (C_1)	July 2019	30 (6)
30 (D_I)	July 2019	25 (5)
Total samples collected		836 (168)

4.2.2 Sample preparation

In order to detect *Campylobacter* spp. and *Arcobacter* spp. cloacal swabs were placed in tubes containing 3 ml of peptone water and vortexed for 1 min. Sample suspensions were processed with a molecular method (family specific PCR), selective methods (ISO 10272-1:2006 and the selective *Arcobacter* spp. method) and a non-selective method (Cape Town Protocol).

65

4.2.3 Cultural isolation of *Campylobacter* species (ISO 10272-1:2006)

The aforementioned sample suspensions (1 ml) were inoculated into Bolton broth (CM0983 - Oxoid, Basingstoke, United Kingdom) (1:10 v/v) supplemented with the corresponding supplement (which contained 10 mg of cefoperazone, 10 mg of vancomycin, 10 mg of trimethoprim and 25 mg of cycloheximide) (SR0183E - Oxoid, Basingstoke, United Kingdom) and 10% horse blood (Medical Research Council, Delft, South Africa), sample suspensions were vortexed. Sample suspensions were incubated at 37ºC for 4-6 h at 42ºC for 44-48 h, for the purpose of resuscitation and enrichment, respectively. Both resuscitation and enrichment occurred under micro-aerophilic conditions achieved by CampyGen sachets (CN00026 - Oxoid, Basingstoke, United Kingdom). After enrichment, a loopful (10 µl) of the enrichment was streaked onto Tryptose Blood Agar (TBA) (CM0233- Oxoid, Basingstoke, United Kingdom) and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (CM0739 - Oxoid, Basingstoke, United Kingdom) supplemented with the corresponding supplement (SR0155E - Oxoid, Basingstoke, United Kingdom) which contained cefoperazone (16 mg) and amphotericin B (5 mg).

4.2.4 Cultural isolation of *Arcobacter* species (Selective *Arcobacter* spp. method)

Due to the isolation of a *Campylobacter*-like organism as detected by the Cape Town protocol (discussed below), a selective method was added for the detection of *Arcobacter* spp. The aforementioned sample suspensions (1 ml) were inoculated into *Arcobacter* broth (CM0965 - Oxoid, Basingstoke, United Kingdom) (1:10 v/v) supplemented with the corresponding supplement (SR0174E - Oxoid, Basingstoke, United Kingdom), sample suspensions were vortexed and then incubated aerobically at 30ºC for 48 h. After incubation, a loopful (10 µl) of the enrichment was streaked onto mCCDA (CM0739 - Oxoid, Basingstoke, United Kingdom) supplemented with the corresponding supplement (SR01745E - Oxoid, Basingstoke, United Kingdom) which contained cefoperazone (4 mg), teicoplanin (2 mg) and amphotericin B (5 mg). Plates were incubated at 30ºC under aerobic conditions for 48 h (Merga *et al.*, 2013).

4.2.5 Cultural isolation of *Campylobacter* and *Arcobacter* species (Cape Town Protocol)

The left-over sample (1 ml) suspension was incubated at 37ºC for 24 h under micro-aerophilic conditions and processed according to the Cape Town protocol as follows: 200 µl of the enrichment was transferred to TBA (CM0233- Oxoid, Basingstoke, United Kingdom) plates which contained a sterile cellulose nitrate filter (0.65 µm and 8 µm pore size) (Sartorius Stedim Biotech, Germany). The enrichment was transferred in a drop wise motion and left to passively pass through the filter at 37ºC for 15-20 min. Thereafter, the filters were aseptically removed from the surface of TBA plates. TBA plates were then incubated for 6 days at 37ºC under micro-aerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) achieved using CampyGen sachets (CN00026 - Oxoid, Basingstoke, United Kingdom). Growth on TBA Plates was monitored every 2-days; at each 2-day interval the CampyGen sachets were replaced.

4.2.6 Confirmation of presumptive *Campylobacter* spp. and *Arcobacter* spp. colonies

After incubation, plates were examined for suspect colonies characterised as being small, round, convex, translucent, whitish/greyish colonies. Suspect *Campylobacter* and *Arcobacter* colonies (four colonies per sample) were streaked onto Columbia blood agar (CBA) (CM0331 - Oxoid, Basingstoke, United Kingdom) enriched with 5% horse blood (Medical Research Council, Delft, South Africa). Typical *Campylobacter* (incubated for 48h at 42°C) and *Arcobacter* (incubated for 48h at 30°C) colonies were tested for genus specific phenotypical and biochemical characteristics such as gram's staining, oxidase, catalase and growth at different temperature/atmosphere combinations. Briefly, pure colonies grown on CBA were suspended on a glass slide and fixed with heat and stained using a commercially available kit (Sigma-Aldrich, St. Louis, United States of America); stained slides were inspected under a microscope, where a pink stain was considered a positive reaction. Oxidase activity was determined by spreading a pure colony on commercially available oxidase strips (Merck, Darmstadt, Germany); the appearance of violet or deep blue colour within 10 sec was seen as a positive reaction. Catalase activity was determined through mixing a pure colony with one drop of hydrogen peroxide solution (3%); a rapid formation of bubbles was considered a positive reaction. Additionally, growth studies were conducted where presumptive *Campylobacter* spp. colonies were streaked on two CBA plates, with the first plate being incubated at 25°C under a micro-aerobic atmosphere for 40 h to 48 h and the second plate incubated at 42°C aerobic conditions for 40 h to 48 h; growth under these conditions was regarded as a negative reaction. Similarly, presumptive *Arcobacter* spp. colonies were streaked on CBA plates and placed under a micro-aerophilic atmosphere at 42°C; growth under these conditions was regarded as a negative reaction.

Confirmed colonies were stored in Microbank™ vials (Davies Diagnostics, Johannesburg, South Africa) with 25 beads (3 mm) with a cryoprotectant liquid (1 ml) containing 10 -15 % glycerol. Aseptic inoculation of Microbank™ vials was according to manufacturer's instructions. Briefly, an inoculating plastic loop was used to pick pure colonies and transferred to Microbank™ vials with cryoprotectant liquid, vials were closed tightly and inverted five times to emulsify the microorganism. In order to allow the microorganism to bind with the beads, the Microbank™ vials were left to stand for 2 min without disturbance, thereafter the cryoprotectant liquid was removed and the Microbank™ vials with isolates were stored at - 80⁰C until molecular analyses could commence.

4.2.7 Molecular confirmation of *Campylobacter* and *Arcobacter* species

Previously preserved colonies were streaked onto CBA (CM0331 - Oxoid, Basingstoke, United Kingdom) and single colonies were suspended in 300 lysis buffer (10 mM Tris-HCl, 1% Triton X-100, 0.5 % TWEEN 20 and 1 mM EDTA) and then boiled (100⁰C) for 10 min. After boiling the bacterial suspension was centrifuged at 5500 x g for 2 min and 250 µl of supernatant was transferred to new Eppendorf tubes with ice cold 99.9% ethanol. Thereafter, the equal parts supernatant and 99.9% ethanol were centrifuged at 13 000 x g for 2 min, the supernatant was discarded, and the pellet was left to dry at room temperature for 1 – 2 h. After drying, 100 µl of 10 mM Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA) was added to the pellet and mixed by vortexing; extracted DNA could be used for PCR amplification using the primer set depicted in Table 4.2. The

PCR reaction mixture (25 µl) contained 12.5 µl 2x *Taq* master-mix (Inqaba Biotec, South Africa) and 0.5 µl of each primer (10 p/mol).

PCR mixtures were subjected to the conditions depicted in Table 4.2 and reactions were performed in a Bio-Rad T100 Thermal Cycler (Bio-Rad, South Africa). A 1.5% agarose gel stained with EZ vision was used for the gel electrophoresis of the PCR products (10 µl). All gels ran for 90 min at 70 V. A 100 bp DNA ladder was used for each run, in order to size the products. A Bio-Rad Gel Doc XR+ System (Bio-Rad, South Africa) and Image Lab Software (version 5.2.1) was used for gel visualisation.

Table 4.2 Primer sequences and PCR conditions for the detection of the *Campylobacteraceae* family, *C. jejuni, C. coli, A. butzleri, A. skirrowii* and *A. cryaerophilus*

Identification	Sequence (5' to 3')	PCR conditions
Family specific primers (Inglis & Kalischuk, 2003)		
16S rRNA 16S rRNA	GGATGACACTTTTCGGAGC CATTGTAGCACGTGTGTC	one cycle of 15 min at 95ºC, 35 cycles each consisting of 30 sec at 94ºC, 90 sec at 58ºC, 1 min at 72°C and a final extension step of 10 min at 72ºC
Species specific primers (Negahdari *et al.*, 2016)		
C. jejuni	CTATTTTATTTTTGAGTGCTTGTG GCTTTATTTGCCATTTGTTTTATTA	one cycle of 2 min at 94ºC, 30 cycles each consisting of 40 sec at 94ºC, 40 sec at 54ºC, 5 min at 72°C and a final extension step of 5 min at 72ºC
C. coli	AATTGAAAATTGCTCCAACTATG TGATTTTATTATTTGTAGCAGCG	
Species specific primers (Soma, 2016)		
A. butzleri	CCTGGACTTGACATAGTAAGAATGA CGTATTCACCGTAGCATAGC	one cycle of 5 min at 94ºC, 30 cycles each consisting of 30 sec at 94ºC, 1 min at 51ºC, 1 min at 72°C and a final extension step of 10 min at 72ºC
A. skirrowii	GGCGATTTACTGGAACACA CGTATTCACCGTAGCATAGC	
A. cryaerophilus	TGCTGGAGCGGATAGAAGTA AACAACCTACGTCCTTCGAC	

4.2.8 Quality control

At each incubation point, control strains: *C. jejuni* subsp. *jejuni* ATCC 29428 (Davies Diagnostics, Johannesburg, South Africa), *C. coli, A. butzleri*, *A. skirrowii* and *A. cryaerophilus* were grown and streaked onto the appropriate agar and incubated under the conditions depicted for ISO 10272-1:2006, selective *Arcobacter* spp. method and the Cape Town protocol. Furthermore, a sterility test was conducted on all prepared plates by incubating plates for 24 h at 37°C, any growth on the plates was taken as a sign of

contamination, and these plates were then discarded. Control strains were used as positive controls for the molecular confirmation and detection of *Arcobacter* spp. and *Campylobacter* spp.

4.2.9 Molecular detection of *Campylobacter* and *Arcobacter* species

In order to determine the presence of *Campylobacter* and *Arcobacter* species using PCR, the left-over (800 µl) enrichment used for the Cape Town protocol was used for DNA extraction; 500 µl increments from swab sample enrichments were pooled as indicated in Table 4.1. To extract DNA the ZymoBIOMICS DNA Miniprep Kit (Inqaba Biotec, South Africa) was used; DNA extractions were according to the manufacturer's instructions. Briefly, 250 µl of enrichment was added to tubes with bashing beads and 750 µl lysis solution and processed with a bead beater for 5 min, after which, the tubes were centrifuged (10 000 x g for 1 min). The resultant supernatant was transferred to a Zymo-spin™ III-F filter in a collection tube and centrifuged (8000 x g for 1 min). To the filtrate collected in the collection tube, 1.2 ml of DNA binding buffer was added and the mixture properly mixed. After which, 800 µl of the mixture was transferred to a Zymo-spin™ IIC-Z column with a collection tube and centrifuged (10 000 x g for 1 min), this step was repeated twice. The Zymo-spin™ IIC-Z column was transferred to a new collection tube and 400 µl DNA wash buffer one was added and centrifuged (10 000 x g for 1 min), the filtrate was discarded. Then, 700 µl of DNA wash buffer two was added to Zymo-spin™ IIC-Z column in a collection tube and centrifuged (10 000 x g for 1 min), the filtrate being discarded. The previous step was repeated but 200 µl of DNA wash buffer two was added. The Zymo-spin™ IIC-Z column was transferred to a micro-centrifuge tube and 100 µl of DNase/RNase was added to the column, and left to incubate for 1 min, and centrifuged (10 000 x g for 1 min). The eluted DNA was transferred to a Zymo-spin™ III-HRC filter in a microcentrifuge tube and centrifuged (16 000 x g for 3 min). After filtration, eluted DNA could be used for Polymerase Chain Reaction (PCR) amplification using the primer sets depicted in Table 4.2.

PCR was performed in 25 µl volumes consisting of 12.5 µl 2x *Taq* master-mix (Inqaba Biotec, South Africa) and 0.5 µl of each primer (10 p/mol) (Table 4.2). All reagents used for the PCR reaction mixture were purchased from Inqaba Biotec, South Africa. PCR mixtures were subjected to the conditions depicted in Table 4.2. PCR was performed in a Bio-Rad T100 Thermal Cycler (Bio-Rad, South Africa). After PCR, gel electrophoresis was conducted using 1.5% agarose gel stained with EZ vision (Inqaba Biotec, South Africa). PCR products (10 µl) and 100 bp DNA ladder were transferred onto wells made on the gel with combs and allowed to run for 90 min at 70 V. Afterwards, the gels were placed and exposed to ultraviolet light in a Bio-Rad Gel Doc XR+ System (Bio-Rad, South Africa) and Image Lab Software (version 5.2.1) was used for gel visualisation.

4.3 Statistical analysis

All statistical analyses were performed using Statistica 13.2 (StatSoft, USA). The data was analysed using a one-way analysis of variance (ANOVA) with season, multi-species farming, water sources, presence of wild water birds and population density as main effects. Homogeneity of variance was determined using Levene's test. Significant differences were determined by least significant means (LSMeans) by using a 95% confidence

interval. If means were significantly different between each subgroup within each category (Table 4.4), post hoc tests were used to determine significance differences.

4.4 Results

4.4.1 Methods and overall prevalence

To determine the prevalence of *Campylobacter* spp. and *Arcobacter* spp., samples were collected from July 2018 to July 2019 and samples were processed with family specific PCR (168 pooled cloacal swabs), ISO 10272-1:2006 (836 cloacal swabs), Cape Town Protocol (836 cloacal swabs) and a selective *Arcobacter* spp. method (415 cloacal swabs). Family specific PCR was employed as the first line of detection, in order to obtain a rapid indication of the presence of species that belong to the *Campylobacteraceae* family. Family specific PCR determined a prevalence of 25.63% (Fig. 4.2). The overall prevalence as determined by each cultural method (Cape Town protocol, ISO 10272-1:2006 and the selective *Arcobacter* spp. method) used for detection of *Campylobacter* spp. and *Arcobacter* spp. is depicted in Figure 4.2. With the ISO 10272-1:2006 and the Cape Town protocol, an average *Campylobacter* spp. (*C. jejuni*: Table 4.3) prevalence of 16.83% and 0% was determined, respectively. Interestingly, if the Cape Town protocol was the only method used, the *Campylobacter* spp. prevalence in the cloacal swabs would have been determined to be 0%. For *Arc*obacter spp. (*A. butzleri*, *A. skirrowii* and *A. cryaerophilus*: Table 4.3) the overall prevalence was determined to be 18.80% and 39.14% by the Cape Town protocol and the selective *Arcobacter* spp. method, respectively; the selective method was more efficient in determining an *Arcobacter* spp. prevalence for this study (Fig. 4.2).
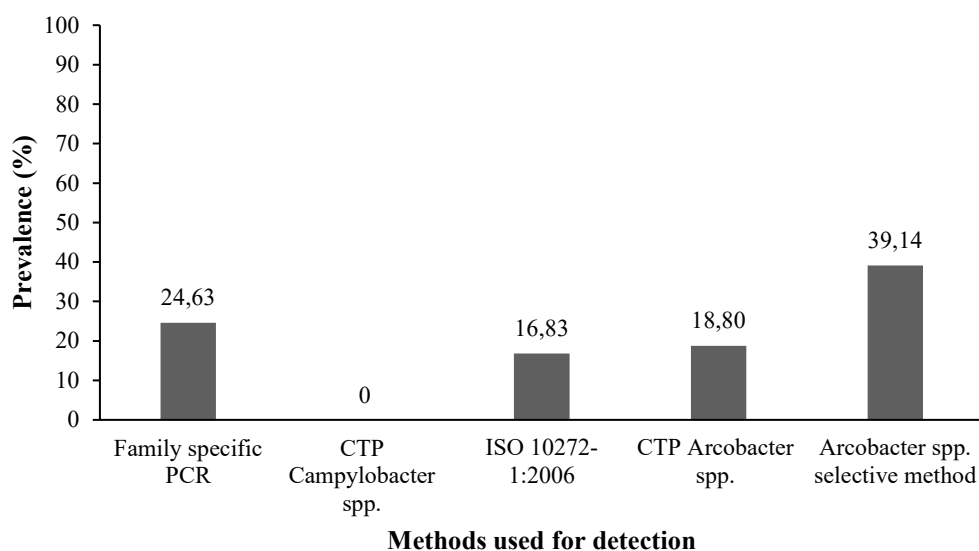
Figure 4.2 Overall prevalence levels as determined by molecular (family specific PCR) and cultural methods (CTP, ISO 10272-1:2006 and the selective *Arcobacter* spp. method)

70

Table 4.3 *Campylobacter* and *Arcobacter* species present at each farm as confirmed by PCR

	C. jejuni	*C. coli*	*A. butzleri*	*A. skirrowii*	*A. cryaerophilus*
Positive farms	B – I, K-M, P-Q, S, V-W, Z-$A_1 – D_1$	-	J, W	S	B, G, J, P – W, Y-D_1

4.4.2 Variation in prevalence levels

While the evaluation of method sensitivity was beyond the scope of this investigation, selective methods were better at detecting *Campylobacter* and *Arcobacter* species (Fig. 4.2). For this reason, prevalence levels determined by the ISO 10272-1:2006 and the selective *Arcobacter* spp. method were used to evaluate the effect of risk factors discussed in the following section.

The samples for this study, were taken during the months of June (2018), July (2018 and 2019), September (2018), November (2018), January (2019), February (2019) and May (2019). These sampling months coincided with winter (June and July), autumn (May), spring (September) and summer (November, January and February). Regarding a possible seasonal influence, prevalence levels were arranged by date of sampling as determined by each method used (Table 4.4). Statistical analysis did not detect any significant differences in prevalence levels for *Campylobacter* spp. ($p = 0.22$) and *Arcobacter* spp. ($p = 0.31$) during winter, spring, autumn and summer (Table 4.4). However, the highest prevalence was seen in spring (26.38%) and autumn (68%) for *Campylobacter* spp. and *Arcobacter* spp., respectively.

It was found that the farms could be split into two livestock categories (farms with ostriches, cattle and sheep and farms with only ostriches: Table 4.4). Prevalence levels for *Campylobacter* spp. ($p = 0.56$) and *Arcobacter* spp. ($p = 0.86$) did not differ significantly. However, prevalence levels for *Campylobacter* spp. (16.11%) and *Arcobacter* spp. (39.00%) were slightly lower for farms with other livestock.

The 30 farms sampled, could be further categorised by the use of either borehole or municipal water (Table 4.4). Farms that made use of borehole water exhibited higher prevalence levels for both *Campylobacter* (23.23%) and *Arcobacter* species (44.50%). However, no significant differences were detected for the prevalence of *Campylobacter* spp. ($p = 0.20$) and *Arcobacter* spp. ($p = 0.24$).

The presence of pests such as rodents and wild birds (farms prone to the presence of wild birds such as doves) can influence prevalence levels. In South Africa ostriches are reared in the open environment, therefore wild birds and rodents were present on all farms (A Olivier 2019, Doctor of Veterinary medicine, South African Ostrich Business Chamber, personal communication, 12 August). However, concerning pests, some farms were more prone to the presence of wild water birds due to the type of environment (Table 4.4). Interestingly, farms not prone to wild water birds exhibited higher prevalence levels for *Campylobacter* spp. (18.14%). Farms prone to wild water birds exhibited higher prevalence levels for *Arcobacter* spp. (44.38%). Differences for the two categories were not significant for *Campylobacter* spp. ($p = 0.60$) and *Arcobacter* spp. ($p = 0.40$).

The farms could be further categorised by the population density (farms with more than 500 ostriches and farms with less than 500 ostriches) of ostriches on the farm, at the time of sampling (Table 4.4). Farms that had more ostriches on the premises exhibited slightly higher prevalence levels for *Campylobacter* spp. (18.38%) when compared to farms that held less ostriches, however the difference was not significant ($p = 0.36$). Farms that held less ostriches on the premises exhibited a higher prevalence of *Arcobacter* spp. (32.00%) when compared to farms that held more ostriches, however statistical differences were not detected ($p = 0.46$).

Table 4.4 Average prevalence of *Campylobacter* spp. and *Arcobacter* spp. from ostriches, as determined by season, multi species farming, water sources, presence of wild water birds, population density

Category	*Campylobacter* spp.		*Arcobacter* spp.	
	Swab samples	Prevalence (%)	Swab samples	Prevalence (%)
Season				
Winter	197 (8)*	14.13	90 (3)	40.67
Spring	224 (8)	26.38	NS[#]	-
Summer	360 (12)	11.17	270 (9)	32.22
Autumn	55 (2)	23.50	55 (2)	68
p-value		0.22		0.31
Multi-species farming				
Farms with ostriches, cattle and/or sheep	751 (27)	16.11	350 (12)	39.00
Farms with only ostriches	85 (3)	23.33	55 (2)	40.00
p-value		0.56		0.86
Water source				
Water from borehole	421 (13)	23.23	265 (8)	44.50
Municipality water	415 (17)	10.44	150 (6)	28.00
p-value		0.20		0.24
Farms prone to the presence of wild water birds				
Wild birds on the farm	421 (16)	15.69	240 (8)	44.38
No wild birds on the farm	415 (14)	18.14	175 (6)	32.17
p-value		0.60		0.40
Population density				
>500 ostriches	197 (8)	18.38	150 (5)	32.00
<500 ostriches	639 (22)	16.27	265 (9)	43.11
p-value		0.36		0.46

*Number of farms in parentheses

[#]Samples not gathered

4.5 Discussion

4.5.1 Method and prevalence levels

Family specific PCR and cultural methods were used to determine the overall prevalence of species that belong to the *Campylobacteraceae* family in cloacal swabs from ostriches. Family specific PCR determined a prevalence of 24.63% (Fig. 4.2). In one other study, the prevalence rate of 6% was determined in faecal samples obtained from 35 ostriches from India using direct PCR (Prince Milton *et al.* 2017). Differences in prevalence levels could be attributed to the fact that the previous study only targeted genes for *Campylobacter* species (namely *C. jejuni*) and made use of a smaller sample size (Prince Milton *et al.* 2017); the present study screened 168 pooled cloacal samples. Nonetheless, both studies are in agreement that species that belong to the *Campylobacteraceae* family can be detected in ostriches with molecular methods.

For the isolation methods used for detection of species that belong to the *Campylobacteraceae* family, numerous broths and agar combinations exist and have been validated for various matrixes such as food, faecal matter as well as swabs (Gharst *et al.*, 2013). However, there is a distinct line that can be drawn between selective and non-selective methods. In this study the selective methods would be the ISO 10272-1:2006 for the detection of *Campylobacter* spp. and the *Arcobacter* spp. selective method developed by Merga *et al.* (2013) and the non-selective method would be the Cape Town protocol. The Cape Town protocol is essentially a passive filtration method and the attention gained by the method can been attributed to the fact that Lastovica & Le Roux (2001) could effectively isolate several *Campylobacter* species, not just the thermotolerant species. For this reason, the Cape Town protocol was used in this study. The results from this study indicate that the Cape Town protocol could not detect *Campylobacter* spp., although it should be noted that gram negative, oxidase and catalase positive microorganisms were detected, however the isolates showed aerotolerance, which deemed the colonies to be presumptive *Arcobacter* spp. colonies. This phenomenon has also been reported by Diergaardt *et al.* (2003), when *Campylobacter*-like microorganisms were isolated and later confirmed to be *Arcobacter* species. Furthermore, research has shown the Cape Town protocol's ability to isolate *Arcobacter* species (Lastovica & Le Roux, 2001). The inability to isolate *Campylobacter* spp. and also the low prevalence level determined for *Arcobacter* spp. by the Cape Town protocol could be attributed to the differences in enrichment periods. For instance, for the ISO 10272-1:2006 method four to six hours is allocated for resuscitation and a further 48 hours for enrichment, whilst for the selective *Arcobacter* spp. method, 48 hours were needed for enrichment. These differences can allow for the postulation that the Cape Town protocol's 24-hour enrichment period was too short for the recovery of stressed and injured cells from cloacal swabs (Hayashi et al., 2013) an aspect that warrants further research.

A prevalence level of 16.83% was determined with the ISO 10272-1:2006 method for *C. jejuni* (Fig. 4.2 and Table 4.3). The presence of thermotolerant *Campylobacter* species is not surprising, as the body temperatures (39 - 41ºC) of avian species are ideal for the survival and growth of *Campylobacter* species whose optimal growth temperature is 42ºC (Allos & Lastovica, 2011). For this study, the prevalence level was higher than the incidence level determined by Ling *et al.* (2011) and Prince Milton *et al.* (2017) but lower than the prevalence levels determined by Oyarzabal *et al.* (1995) and Cuomo *et al.* (2007) in ostriches. As noted by

other researchers, comparisons between studies can be difficult, for instance, when this study was compared to the previous research mentioned above, studies differed by sample number, sample type and analytical method used. More specifically, Ling *et al.*, (2011) examined 31 cloacal swabs, Prince Milton *et al.* (2017) made use of only PCR to determine prevalence and instead of using mCCDA, Oyarzabal *et al.* (1995) made use of a selective chromogenic agar; Campy-cefex.

Reports on the detection of *Arcobacter* spp. in food-producing animals is sparse and even though some researchers have proven that poultry species are reservoirs of *Arcobacter* spp. at prevalence levels of 4-15%, 38%, 26.14% and 16.7% for turkey, ducks and geese, respectively (Shange *et al.*, 2019), it seems as if not all poultry species used for human consumption have been studied; in fact, this is the first reported evidence of *Arcobacter* spp. (*A. butzleri*, *A. skirrowii* and *A. cryaerophilus*: Table 4.3) in live ostriches. To determine the prevalence of *Arcobacter* spp., the Cape Town protocol and a selective method were used, which determined a prevalence of 18.80% and 39.14%, respectively (Fig 4.2). For this study, the presence of *Arcobacter* spp. was an interesting outcome as even though *Arcobacter* spp. have been isolated from other poultry species, research is still unclear on whether *Arcobacter* species form part of the intestinal flora of avian species, due to the optimum growth temperature requirement of 15-25ºC. Nonetheless, the findings from this study can contribute to the conversation of whether *Arcobacter* species are commensal microorganisms of the avian gastrointestinal tract or transient colonisers as noted by others (Adesiji *et al.*, 2011).

4.5.2 Variation in prevalence levels

In literature, certain risk factors have been identified that influence the prevalence of species that belong to the *Campylobacteraceae* family at primary production. These risk factors include: season, multi-species farming, contaminated water sources, the presence of pests (rodents, migratory birds and wild water birds) on the farm premises, hygiene practices in place, contaminated feed and population density (Shange *et al.*, 2019; Huneau-Salaün *et al.*, 2009).

For this study (Table 4.4), a higher prevalence of *Campylobacter* spp. was seen during the warmer spring month of September. Higher prevalence levels during the warmer months are expected as previous research indicates that sampling during the warmer parts of the year can result in determining a higher prevalence in species that belong to the *Campylobacteraceae* family. For *Campylobacte*r spp. this phenomenon has been reported in broiler chickens (Weber *et al.*, 2014; Smith *et al.*, 2016); Kalupahana *et al.*, 2018), ducks and geese (Çelİk *et al.*, 2018). The higher prevalence levels during the warmer parts of the year has been attributed to indirect temperature dependant factors such as an increase in rodents, insects and presence of migratory birds, which aid in horizontal transmission. Even though this notion is not fully explored, it is also speculated that *Campylobacter* spp. thrive and survive better during the warmer parts of the year. However, a peak during summer was not witnessed (Table 4.4), this could be attributed to the fact that during the summer of 2018 and 2019, the study area was experiencing extremely high temperatures (>32ºC) and a drought. Therefore, the hot and dry conditions could have created a non-conducive environment for the survival of *Campylobacter* spp., a similar occurrence was witnessed during a hot and dry Switzerland summer (Ring *et al.*, 2005). A high *Arcobacter* spp. prevalence was seen during autumn (Table 4.4); in contradiction to previous

studies that reported an increase in prevalence levels as the environmental temperature increases (Andersen *et al.*, 2007; Kalupahana *et al.*, 2018). The higher prevalence during the cooler (<22ºC) months of the year could be attributed to *Arcobacter*'s ability to grow at low temperature ranges rather than high temperature ranges like *Campylobacter* spp. (Ramees *et al.*, 2017).

Other livestock such as cattle and sheep have been reported to be reservoirs of species that belong to the *Campylobacteraceae* family. *Campylobacter* spp. prevalence has been reported to range from 13-69% for cattle and 14-25% for sheep. Additionally, for *Arcobacter* spp., prevalence levels ranging from 8-43% and 10.1-18.5% for cattle and sheep, respectively have been reported (Shange *et al.*, 2019). The presence of other livestock at a poultry farm increases the number of hosts for species that belong to the *Campylobacteraceae* family and these can be transferred to flocks by alternative vehicles such as insects; subsequently increasing the likelihood of a flock obtaining an infection (Wagenaar *et al.*, 2006). It should be noted that this influence has been studied extensively in relation to broiler chickens, and by extension the conventional broiler chicken farming system. Therefore, when other farming systems (and other poultry species) are evaluated, the presence of other livestock might not have the same effect. As for this study, rearing of ostriches on a farm with other livestock did not significantly increase the prevalence of *Campylobacter* spp. and *Arcobacter* spp. This is attributed to the typical biosecurity measures implemented at each farm as mandated by South African VPN 04/2012. For instance, the slightly lower prevalence could be an indication of the fact that ostriches from registered farms are typically separated from other livestock; other livestock are generally not permitted any access to ostriches in order to stop the spread of recognised diseases within the ostrich industry, such as salmonellosis (Scholtz, 2014). In this regard, the ostrich industry has stringent rules that govern the contact of ostriches with other livestock, but not necessarily pests such as rodents.

Contaminated water sources can also aid horizontal transmission; water sources can be contaminated with species that belong to the *Campylobacteraceae* family, as *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* have been detected in water troughs located on sheep and cattle farms (Giacometti *et al.*, 2015). For this study, the use of municipal water showed lower levels of colonisation of *Campylobacter* spp. and *Arcobacter* spp. compared to farms that made use of underground borehole water (Table 4.4). It should be noted that the municipal water is the same potable water used for human consumption that would indicate that the municipal water had the superior microbiological quality. A similar result was noted when the prevalence of *Arcobacter* spp. was investigated in market weight turkeys, as the use of city water correlated with the absence of *Arcobacter* spp. in the turkey flocks (Andersen *et al.*, 2007). Furthermore, when the state of ground water from South Africa was explored, it was reported that borehole water often exceeds the microbial limits as depicted in SANS 241-1:2015 (Esterhuizen *et al.*, 2012; Baloyi & Diamond, 2019).

Pests such as migratory birds and rodents are important vehicles that aid horizontal transmission as these wild animals can carry species that belong to the *Campylobacteraceae* family in their gastrointestinal tract (Colles *et al.* 2008; Hamidi, 2018) and can introduce these species to the soil and water (environment). Pests tend to be a risk for housed food-producing animals such as broiler chickens and pigs, although food-producing animals reared in free-range systems can have frequent contact with migratory birds and rodents (A Olivier 2019, Doctor of Veterinary medicine, South African Ostrich Business Chamber, personal

communication, 12 August). However, for this study from the information gathered from each farm, some farms are more prone to the presence of wild water birds due to wet farm environments. When the influence of wild water birds was analysed, a higher prevalence for *Arcobacter* species occurred (Table 4.4); it is postulated that the additional presence of wild water birds increased the number of hosts of *Arcobacter* spp. at primary production, and subsequently increased the likelihood of environmental contamination and the introduction of *Arcobacter* spp. to flocks through horizontal transmission, as seen for other infections (Olivier, 2014). In this case, higher prevalence levels were seen for only *Arcobacter* spp., this could suggest that wild water birds carried mostly *Arcobacter* species or *Campylobacter* species not confirmed with PCR, as seen for other wild birds (Johansson *et al.*, 2018).

When ostrich population densities were compared, no statistical differences were detected (Table 4.4); this contradict the notion that intra-flock transmission of an infection can be increased by high population densities (Olivier, 2014; Sakaridis *et al.*, 2018) as an increased flock size increases susceptible hosts for an infection (Huneau-Salaün *et al.*, 2009). However, it should be noted that when population densities in relation to *Campylobacter* infection have been studied, inconclusive results have also been found (Newell *et al.*, 2011). For this study, the lack of statistical differences can potentially be associated with the fact that population densities are recognised and mitigated well in the industry, as it is recognised that high stoking densities can result in slow growth rates, disease build up, pecking and cannibalism within an ostrich flock (Shanawany & Dingle, 1999); outcomes that can affect the profitability of a flock.

4.6 Conclusion

Overall, this study provides evidence that ostriches reared in South Africa can be reservoirs of both *Campylobacte*r spp. and *Arcobacter* spp. This is the first study to determine *Arcobacter* species in live ostriches from South Africa. This study also provided evidence that selective detection methods showed higher prevalence levels; this occurrence is attributed to the longer enrichment periods, which potentially allowed for a more efficient resuscitation of injured and/or stressed cells. Additionally, prevalence levels were influenced by season, the presence of wild water birds and the source of water. Therefore, future research should aim to include environmental sampling, to determine possible genetic relatedness between *Campylobacter* spp. and *Arcobacter* spp. isolates from ostriches and possible vehicles and/or vectors that can aid horizontal transmission. Future research should also include the study of *Campylobacter* spp. and *Arcobacter* spp. contamination in ostrich meat, as the consumption of contaminated meat will ultimately affect human health.

4.7 References

Acheson, D. & Allos, B.M. (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. *Clinical Infectious Diseases*, **32**, 1201–1206.

Adesiji, Y.O., Coker, A.O. & Oloke, J.K. (2011). Detection of *Arcobacter* in feces of healthy chickens in Osogbo, Nigeria. *Journal of Food Protection*, **74**, 119–121.

Allos, B.M. & Lastovica, A.J. (2011). *Campylobacter* Infections. In: *Tropical Infectious Diseases: Principles, Pathogens and Practise* (edited by R. L. Guerrant, D.H. Walker, & P.F. Weller). Pp. 145-149. Philadelphia: Saunders/Elsevier

Alonso-Calleja, C., Martínez-Fernández, B., Prieto, M. & Capita, R. (2004). Microbiological quality of vacuum-packed retail ostrich meat in Spain. Food Microbiology, 21, 241–246.

Andersen, M.M.E., Wesley, I. V., Nestor, E. & Trampel, D.W. (2007). Prevalence of *Arcobacter* species in market-weight commercial turkeys. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, **92**, 309–317.

Baloyi, R.S. & Diamond, R.E. (2019). Variable water quality of domestic wells emphasizes the need for groundwater quality monitoring and protection: Stinkwater, Hammanskraal, Gauteng. *Water SA*, **45**, 216.

Banting, G. & Figueras S.M.J. 2017. Global Water Pathogen Project. URL http://www.waterpathogens.org/book/*Arcobacter*. 10/08/2019.

Brand, T. (2006). Volstruisvoedingsriglyne. In: *Volstruishandleiding* (edited by C. Muller)*.* Pp. 42. Western Cape: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Çelİk, E., Sağlam, A.G., Çelebİ, Ö. & Otlu, S. (2018). Isolation of *Arcobacter* spp. from domestic ducks and geese and identification of the recovered isolates by using molecular method. *Turkish Journal of Veterinary and Animal Sciences*, **42**, 1–6.

Coker, A.O., Smith, R.D., Oyelese, B.N. Onipede, K.O. & Zailani, C.L (2002). Human campylobacteriosis in developing countries. *Emerging Infectious Diseases*, **8**, 237–243.

Collado, L., Gutiérrez, M., González, M. & Fernández, H. (2013). Assessment of the prevalence and diversity of emergent *Campylobacteria* in human stool samples using a combination of traditional and molecular methods. *Diagnostic Microbiology and Infectious Disease*, **75**, 434–436.

Cuomo, A., Dipineto, L., Santaniello, A., Matteoli, G., Sarli, T., Vecchia, D. Della, Fioretti, A. & Menna, L.F. (2007). Detection of thermotolerant *Campylobacter* in ostriches (*Struthio camelus*) in Italy. *Veterinary Journal*, **174**, 439–441.

Diergaardt, S.M., Venter, S.N., Chalmers, M., Theron, J. & Brozel, V.S. (2003). Evaluation of the Cape Town Protocol for the isolation of *Campylobacter* spp. from environmental waters: short communication. *Water SA*, **29**, 225–229.

Elmali, M. & Can, H.Y. (2016). Occurrence and antimicrobial resistance of *Arcobacter* species in food and slaughterhouse samples. *Food Science and Technology (Campinas)*, **37**, 280–285.

Esterhuizen, L., Fossey, A. & Lues J. F.R. (2012). Dairy farm borehole water quality in the greater Mangaung region of the Free State province, South Africa. *Water SA,* **38**, 803–6.

European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2017). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal*, **15**, 1–227.

European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA Journal,* **13**, 1-231.

Evers, E.G., van der Fels-Klerx, H.J., Nauta, M.J., Schijven J.F. & Havelaar, A.H. (2008). *Campylobacter* source attribution by exposure assessment. *International Journal of Risk Assessment and Management*, **8**, 174–189.

Geissler, A.L., Carrillo, F. B., Swanson, K., Patrick, M. E., Fullerton, K. E., Bennett, C., Barrett, K. & Mahon, B. E. (2017). Increasing *Campylobacter* infections, outbreaks, and antimicrobial resistance in the United States, 2004-2012. *Clinical Infectious Diseases* **65**, 1624–31.

Gharst, G., Oyarzabal, O.A. & Hussain, S.K. (2013). Review of current methodologies to isolate and identify *Campylobacter* spp. from foods. *Journal of Microbiological Methods*, **95**, 84–92.

Giacometti, F., Lucchi, A., Francesco, A. Di, Delogu, M., Grilli, E., Guarniero, I., Stancampiano, L., Manfreda, G., Merialdi, G. & Serraino, A. (2015). *Arcobacter butzleri*, *Arcobacter cryaerophilus*, and *Arcobacter skirrowii* circulation in a dairy farm and sources of milk contamination. *Applied and Environmental Microbiology*, **81**, 5055–5063.

Girolami, A., Marsic, I. D'Andrea, G., Braghieri, A., Napolitano, F. & Cifuni., G.F. (2003). Fatty acid profile, cholesterol content and tenderness of ostrich meat as influenced by age at slaughter and muscle type. *Meat Science*, **64**, 309-315.

Hald, T., Aspinall, W., Devleesschauwer, B., Cooke, R., Corrigan, T., Havelaar, A.H., Gibb, H.J., Torgerson, P.R., Kirk, M.D., Angulo, F.J., Lake, R.J., Speybroeck, N. & Hoffmann, S. (2016). World Health Organization estimates of

the relative contributions of food to the burden of disease due to selected foodborne hazards: A structured expert elicitation. *PLoS ONE*, **11**, 1–35.

Hamidi, K. (2018). How do rodents play role in transmission of foodborne diseases? *Nutrition & Food Science International Journal*, **6**, 10–13.

Hayashi, M., Kubota-Hayashi, S., Natori, T., Mizuno, T., Miyata, M., Yoshida, S., Zhang, J., Kawamoto, K., Ohkusu, K., Makino, S. & Ezaki, T. (2013). Use of blood-free enrichment broth in the development of a rapid protocol to detect *Campylobacter* in twenty-five grams of chicken meat. *International Journal of Food Microbiology*, **163**, 41–46.

Heredia, N. & García, S. (2018). Animals as sources of food-borne pathogens: A review. *Animal Nutrition Journal*, **2018**, 4–9.

Hoffman, L. C. & Mellett, F. D. (2003). Quality characteristics of low-fat ostrich meat patties formulated with either pork lard or modified corn starch, soya isolate and water. *Meat Science*. **65**, 869–75.

Hoffman, L.C. & Fisher, P. (2001). Comparison of meat quality characteristics between young and old ostriches. *Meat Science*, **59**, 335–337.

Huneau-Salaün, A., Chemaly, M., Bouquin, S. Le, Lalande, F., Petetin, I., Rouxel, S., Michel, V., Fravalo, P. & Rose, N. (2009). Risk factors for *Salmonella enterica* subsp. *enterica* contamination in 519 French laying hen flocks at the end of the laying period. *Preventive Veterinary Medicine*, **89**, 51–58.

Inglis, G.D. & Kalischuk, L.D. (2003). Use of PCR for direct detefction of *Campylobacter* species in bovine feces. *Applied and Environmental Microbiology*, **69**, 3435–3447.

ISO 10272:1 (2006). Microbiology of food and animal feeding stuffs horizontal method for detection and enumeration of *Campylobacter* spp. URL https://www.iso.org/standard/37091.html. 03/01/2017.

Johansson, H., Ellström, P., Artursson, K., Berg, C., Bonnedahl, J., Hansson, I., Hernandez, J., Lopez-Martín, J., Medina-Vogel, G., Moreno, L., Olsen, B., Engvall, E.O., Skarin, H., Troell, K., Waldenström, J., Ågren, J. & González-Acuña, D. (2018). Characterization of *Campylobacter* spp. isolated from wild birds in the antarctic and Sub-Antarctic. *PLoS ONE*, **13**, 1–12.

Jorgensen, P. (2014). Introduction. In: Ostrich manual (edited by P. Jorgensen). Pp 6-9. Western Cape: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, **28**, 687–720.

Kalupahana, R.S., Kottawatta, S.A. & Somarathne, S. (2018). Weather correlates of *Campylobacter* prevalence in broilers at slaughter under tropical conditions in Sri Lanka. *Epidemiology and Infection*, **2018**, 1-8.

Lastovica, A.J. & Le Roux, E. (2001). Efficient isolation of *Campylobacter upsaliensis* from stools. *Journal of Clinical Microbiology*, **39**, 4222–4223.

Lastovica, A.J., On, L.W. & Zhang, L. (2014). The Family *Campylobacteraceae*. In: *The prokaryotes-Deltaproteobacteria and Epsilonproteobacteria* (edited by E. Rosenberg *et al.*). Pp. 308 – 326. Berlin: Springer-Verlag.

Ling, Y.E., Aziz, S.A. & Abu, J. (2011). Occurrence of *Campylobacter* and *Salmonella* spp. in Ostrich. *J. Vet. Malaysia*, **24**, 6–8.

Merga, J.Y., Williams, N.J., Miller, W.G., Leatherbarrow, A.J.H., Bennett, M., Hall, N., Ashelford, K.E. & Winstanley, C. (2013). Exploring the diversity of *Arcobacter butzleri* from cattle in the UK using MLST and whole genome sequencing. *PLoS ONE*, **8**, 1–12.

Negahdari, B., Shirazi, M., Malekshahi, Z.V., Kadkhodazedeh, M., Hajikhani, S. & Rahmati, M. (2016). Identification of *Campylobacter Jejuni* and *Campylobacter coli* from diarrheic samples using PCR. *International Journal of Health Studies*, **2**, 1–3.

Newell, D.G. & Fearnley, C. (2003). Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology*, **69**, 4343–4351.

Newell, D.G., Elvers, K.T., Dopfer, D., Hansson, I., Jones, P., James, S., Gittins, J., Stern, N.J., Davies, R., Connerton, I., Pearson, D., Salvat, G.S. & Allen, V.M. (2011). Biosecurity-based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. *Applied and Environmental Microbiology*, **77**, 8605–8614.

Olivier, A.J. (2014). Health management. In: Ostrich manual (edited by P. Jorgensen). Pp 86-107. Western Cape: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Oyarzabal, O.A., Donald, E.C. & Hoerrb, F.J. (1995). Incidence of *Campylobacters* in the intestine of avian species in Alabama. *Avian Diseases*, **39**, 147–151.

Platts-Mills, J.A. & Kosek, M. (2014). Update on the burden of *Campylobacter* in developing countries. *Current Opinion in Infectious Diseases*, **27**, 444–450.

Prince Milton, A.A., Agarwal, R.K., Priya, G.B., Saminathan, M., Aravind, M., Reddy, A., Athira, C.K., Anjay, Ramees, T.P., Dhama, K., Sharma, A.K. & Kumar, A. (2017). Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in captive wildlife species in India. *South Afr J Epidemiol Infect J Epidemiol Infect*, **18**, 177–182.

Ramees, T.P., Rathore, R.S., Bagalkot, P.S., Mohan, H.V., Kumar, A. & Dhama, K. (2014). Detection of *Arcobacter butzleri* and *Arcobacter cryaerophilus* in clinical samples of humans and foods of animal origin by cultural and multiplex PCR based methods. *Asian Journal of Animal and Veterinary Advances*, **9**, 243–252.

Ring, A.M., Zychowska, M.A., Stephan, R., Ring, M., Zychowska, M.A. & Stephan, R.A. (2005). Dynamics of *Campylobacter* spp. spread investigated in 14 broiler flocks in Switzerland. *Avian Diseases*, **49**, 390–396.

Sakaridis, I., Ellis, R.J., Cawthraw, S.A., Vliet, A.H.M. van, Stekel, D.J., Penell, J., Chambers, M., Ragione, R.M. La & Cook, A.J. (2018). Investigating the association between the caecal microbiomes of broilers and *Campylobacter* burden. *Frontiers in Microbiology*, **9**, 1–9.

Samie, A., Obi, C.L., Barrett, L.J., Powell, S.M. & Guerrant, R.L. (2007). Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: Studies using molecular diagnostic methods. *Journal of Infection*, **54**, 558–566.

Scholtz, A.J. (2014). Biosecurity in practice. In: Ostrich manual (edited by P. Jorgensen). Pp 108-120. Western Cape: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Shanawany, M. M. & Dingle, J. (1999). Ostrich production systems. In: FAO animal production and health paper. Pp. 144. Rome: Food and Agriculture Organization of the United Nations.

Shange, N., Gouws, P. & Hoffman, L.C. (2019). *Campylobacter* and *Arcobacter* species in food - producing animals: prevalence at primary production and during slaughter. *World Journal of Microbiology and Biotechnology*, **35**, 146.

Skarp, C.P.A., Hänninen, M.L. & Rautelin. H.I.K. (2016). Campylobacteriosis: The role of poultry meat. *Clinical Microbiology and Infection,* **22**, 103–9.

Smith, K., Reimers, N., Barnes, H.J., Lee, B.C., Siletzky, R. & Kathariou, S. (2004). *Campylobacter* colonization of sibling turkey flocks reared under different management conditions. *Journal of food protection*, **67**, 1463–8.

Soma, S.M. (2016). *Studies on Arcobacter species of animal and human origin.* MVSc Thesis. Sri Venkateswara Veterinary University, India

Stanley, K. & Jones, K. (2003). Cattle and sheep farms as reservoirs of *Campylobacter*. *Journal of applied microbiology*, **94**, 104S–113S.

Tully, T.N. & Shane, S.M. (2016). Husbandry practices as related to infectious and parasitic diseases of farmed ratites. *Revue Scientifique et Technique de l'OIE*, **15**, 73–89.

Umar, S., Maiyah, A.T., & Mushtaq, A. (2016). *Campylobacter* infections in poultry: update on challenges and potential immune interventions. *World's Poult. Sci. Association*, **72**, 381–90.

Vandeplas, S., Dauphin, R.D. & Palm, R. (2010). Prevalence and sources of *Campylobacter* spp. contamination in free-range broiler production in the southern part of Belgium. *Biotechnol. Agron. Soc. Environ.*, **14**, 279–288.

Van Driessche E., Houf, K., Vangroenweghe, F., De Zutter, L. & Van Hoof. J. (2005). Prevalence, enumeration and strain variation of *Arcobacter* species in the faeces of healthy cattle in Belgium. *Veterinary Microbiology,* **105**, 149–154.

Van Driessche E., Houf, K., Vangroenweghe, F., Nollet, N., De Zutter, L., Vandamme, P. & Van Hoof, J. (2004). Occurrence and strain diversity of *Arcobacter* species isolated from healthy Belgian pigs. *Research in Microbiology,* **155**, 662–666.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N., Zissis,G. & Butzler, J. (2004). *Arcobacter* species in humans. *Emerging Infectious Diseases*, **10**, 1863-1867.

Veterinary Procedural Notices (VPN) 04 (2012). Standard for the requirements, registration, maintenance of registration and official control of ostrich compartments in South Africa. URL https://www.nda.agric.za. 25/07/2019.

Veterinary Procedural Notices (VPN) 52 (2018). Guidelines on the verification of compliance with process hygiene control and microbiological food safety performance objectives under the Meat Safety Act. URL at http://www.rmaa.co.za/wp-content/uploads/2018/12/VPN-52-Microbiological-Reference-Criteria-for-Meat-19-October-2018-Draft.pdf. 5/08/2019.

Wagenaar, J. a, Mevius, D.J. & Havelaar, A. H. (2006). *Campylobacter* in primary animal production and control strategies to reduce the burden of human campylobacteriosis. *Revue scientifique et technique (International Office of Epizootics)*, **25**, 581–94.

Weber, R., Auerbach, M., Jung, A. & Glünder, G. (2014). *Campylobacter* infections in four poultry species in respect of frequency, onset of infection and seasonality. *Berliner und Münchener tier ärztliche Wochenschrift*, **127**, 257–26.

CHAPTER 5

Prevalence of *Campylobacter* and *Arcobacter* species on ostrich carcasses during processing

Abstract

Two approaches were used to assess *Campylobacter* spp. and *Arcobacter* contamination on ostrich carcasses during slaughter. For the first study, meat samples were obtained post-skinning (n = 30), post-evisceration (n = 30) and post-chilling (n = 30) from the same carcases on one day. Selective cultural methods were used to detect *Campylobacter* and *Arcobacter* species. At post-skinning, *Arcobacter* spp. was detected at a prevalence level of 73%. At post-evisceration 73% and 83% of samples were contaminated with *Campylobacter* spp. and *Arcobacter* spp., respectively. At post-chilling, a slight reduction in contamination was seen, as 66% and 67% of samples collected were contaminated with *Campylobacter* spp. and *Arcobacter* spp., respectively. The second study aimed to determine the occurrence of *Campylobacter* spp. and *Arcobacter* spp. if routine testing was required for abattoirs. Meat samples were obtained post-evisceration (before chilling) on a weekly basis from three export approved abattoirs situated in the Western Cape (n = 305). *Campylobacter* spp. and *Arcobacter* spp. was detected with PCR and cultural methods. Additionally, *E. coli* and coliforms were enumerated to determine the occurrence of faecal contamination during slaughter. Overall, a low occurrence of *Campylobacter* spp. (0.98% and 0%), *Arcobacter* spp. (1.31% and 1.64%), *E. coli* (0.13 $\log_{10}$ cfu/g) and coliforms (0.53 $\log_{10}$ cfu/g) were determined for all three abattoirs. No relationship was seen between the occurrence of species belonging to the *Campylobacteraceae* family and faecal indicators. The first study demonstrates that contamination can occur during the slaughter process, but when good hygiene practices are adhered to; low prevalence levels can be achieved, as seen in the second study.

5.1 Introduction

In South Africa, meat safety practices are governed by the Veterinary Procedural Notices (VPN) 52/2018. This procedural notice derives its mandate for meat safety from the Meat Safety Act (Act No. 40 of 2000). This VPN provides slaughter facilities with specific hygiene practices that should be adhered to, as well as specific microbiological specifications that classify meat products (from bovine, equine, game, ovine, porcine, caprine, ostrich, crocodile, chicken and rabbit) as satisfactory, acceptable and unsatisfactory. Microbiological specifications are provided for indicator microorganisms (aerobic bacteria, *Enterobacteriaceae* and *Escherichia coli* (*E. coli*)) and certain pathogens such as Shiga toxin *E. coli* (STEC), *Salmonella, Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens* and *Yersinia enterocolitica*. However, specifications for species belonging to the *Campylobacteraceae* family are not included within the VPN 52/2018.

Species that belong to the *Campylobacteraceae* family are of importance to public health. For instance, *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) have been identified as the main cause of gastro-intestinal infections known as campylobacteriosis and the cause of Guillian Barré syndrome in humans (Kaakoush *et al.*, 2015). *Campylobacter* cells ranging from 500 to 800 cfu are able to cause campylobacteriosis (Robert *et al.*, 1988; Keener *et al.*, 2004). Additionally, *Arcobacter* species such as *Arcobacter butzleri* (*A. butzleri*) have been identified as an emerging pathogen (Ramees *et al.*, 2017), a hazard to human health

(Vandenberg *et al.*, 2004), the cause of gastrointestinal infections and severe diseases such as bacteraemia and septicaemia (Collado & Figueras, 2011). It is also noted in literature that in immunocompromised individuals, infections caused by species belonging to the *Campylobacteraceae* family tend to be more severe and persistent (Samie *et al.*, 2007). Species that belong to the *Campylobacteraceae* family can be transferred to humans through various routes such as contact with infected humans, infected animals and consumption of contaminated water (Evers *et al.*, 2008). However, the aforementioned routes are secondary to the consumption of contaminated meat and meat products (Collado & Figueras, 2011; Kaakoush *et al.*, 2015).

At primary production species that belong to the *Campylobacteraceae* family can colonise the gastrointestinal tract of food-producing animals such as cattle, sheep, pigs and poultry (including ostriches as seen in Chapters 3 and 4) as reviewed by Shange *et al.* (2019). Successful colonisation at primary production can in turn result in the contamination of carcasses and by extension meat and meat products, if the slaughter process is not conducted in a hygienic manner (De Smet *et al.*, 2010). Research to evaluate the slaughter process in relation to species belonging to the *Campylobacteraceae* family, has shown that certain steps such as skinning and evisceration can aid the spread of *Campylobacteraceae* species through faecal contamination of sterile carcass surfaces (Berrang & Dickens, 2000). Also, deboning steps can possibly help spread contamination (Gill & Harris, 1982; Gouws *et al.*, 2017). Even though studies that evaluate the ostrich process in relation to *Campylobacter* and *Arcobacter* species are scarce, the possibility of faecal contamination occurring during the slaughter of ostriches has been proven (Karama *et al.,* 2003). Faecal contamination is a major contributor to *Campylobacter* spp. and *Arcobacter* spp. being present on carcass surfaces as these species are found in the gastrointestinal tract of food-producing animals (Shange *et al.*, 2019). Karama *et al.* (2003) found that faecal contamination of ostrich carcasses occurred during skinning and evisceration. Faecal contamination during skinning was attributed to hands previously in contact with the skin contacting carcass flesh. The prevalence of carcasses contaminated with faecal matter was highest during evisceration, even though a reason was not given by Karama *et al.* (2003), it could be speculated that the cause was the rapture of the viscera and/or spillage of intestinal fluid during evisceration. The information reported by Karama *et al.* (2003) is insightful, however, it should be mentioned that faecal contamination was determined through the enumeration of a faecal indicator microorganism; such as *E. coli*. Currently, there is a dearth of information regarding the possible contamination of ostrich carcasses with species belonging to *Campylobacteraceae* family and by extension a lack of regulatory information that can help govern the presence and/or the contamination of ostrich carcasses (and ostrich products) with *Campylobacter* spp. and *Arcobacter* spp. Therefore, to mitigate the lack of research in this area the present study was designed to determine the distribution and prevalence of *Campylobacter* and *Arcobacter* species during the slaughter of ostriches.

5.2 Materials and method

5.2.1 Abattoir

For this study samples were obtained from export approved abattoir(s) and slaughter occurred as follows: rested (approximately 24 h of lairage) slaughter-aged ostriches were brought into slaughter facilities and stunned. After stunning, unconscious ostriches were hung by both legs and exsanguinated. Exsanguination included making an incision across the throat and inserting the knife at the centre of neck at the depression of

the breastbone, the knife was then pushed upwards in order to effectively sever the anterior vena cava (Hoffman *et al.,* 2012). After bleeding, feathers were manually plucked. The head was removed at its occipito-atlantal articulation. Ostriches were then flayed (skinned) by making a longitudinal ventral incision from the neck to the cloaca and horizontal incisions across the abdomen to the tibio-tarsal joint, the hide was manually pulled off, and further incisions were made to loosen the hide. Ostriches were then reversed and hooked by the wings (Fig. 5.1). Feet were removed by severing the tibio-tarsal joint. To eviscerate carcasses, a longitudinal ventromedial incision was made from the breast to the abdomen, and abdominal organs were removed, and the thoracic cavity was emptied. After evisceration carcasses were quartered and thighs were chilled for 24 h (0 - 4°C) (Hoffman *et al.*, 2010).
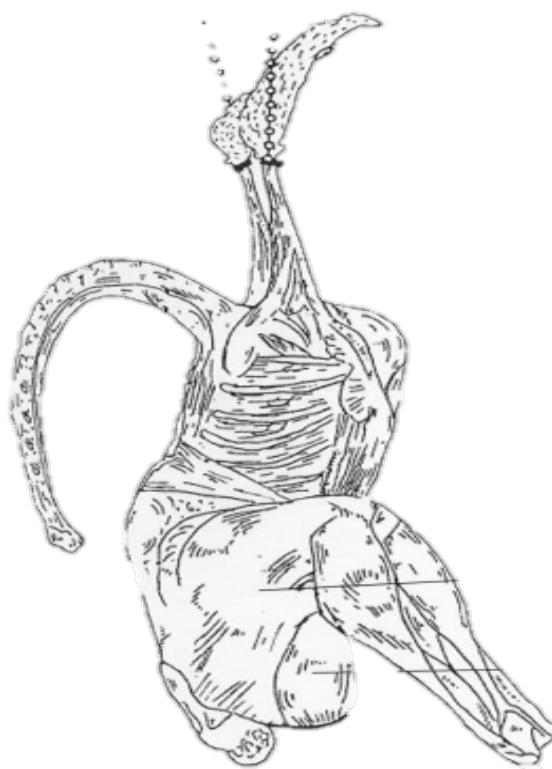
Figure 5.1 Final carcass position at the end of the slaughter process (VPN 52/2018)

5.2.2 Sample collection

Distribution of Campylobacter and Arcobacter species during slaughter (first study)

In the fourth Chapter of this thesis, cloacal swabs were collected from ostriches from 30 different farms situated in the Oudtshoorn area. Ostriches from one of the farms with the highest *Campylobacter* spp. and *Arcobacter* spp. prevalence were sampled during slaughter for this Chapter.

To determine points of contamination during slaughter, ostrich carcasses were sampled during the month of February (2019). Sampling took place at an export approved abattoir, located in the Western Cape region with a capacity of 800 birds per hour (MERC, 2010). For this study, the same 30 carcasses were sampled at each slaughter stage, namely post-skinning (stage 1), post-evisceration (stage 2) and post-chilling (stage 3;

post-chill samples were collected the following day before deboning commenced). Overall, 90 samples (30 samples x 3 slaughter stages/sampling points = 90 samples) were obtained for this trial. It should be noted that ostriches from various farms were slaughtered and ostriches used for this study, were not slaughtered first, therefore sampling commenced after 10h00 (after tea-time).

In order to determine contamination, the inner thigh area was sampled at each sampling point, as this was the mostly likely area to be contaminated with faecal matter (Karama *et al.*, 2003). At each slaughter stage, namely post-skinning, post-evisceration and post-chilling, 10 g (an area of 25-30 cm^2) was excised from the inner thigh area, using sterile forceps and scalpel. Forceps and scalpel were dipped in ethanol and flamed before incisions were made to the thighs, to ensure that sampling was conducted in an aseptic manner. Individual samples were placed in stomacher bags, air was expelled before the bags were sealed and placed in polystyrene containers with ice. Chilled samples were transported to the Food Science department (Stellenbosch University).

Prevalence of Campylobacter and Arcobacter spp. during slaughter (second study)

In order to obtain a realistic view of *Campylobacter* and *Arcobacter* spp. contamination at slaughter, during the month of May and June (2019) samples were obtained from three ostrich abattoirs (Abattoir A, B and C with a capacity of 800, 400 and 200 birds per hour, respectively (MERC, 2010)) situated in the Western Cape and Eastern Cape. In total 305 samples were obtained over a 5-week sampling period (Table 5.1). Sampling only occurred post-evisceration (or prior to final chilling), as this is the recommended area of sampling as depicted by VPN 52/2018. The aforementioned (first study) sampling and transport procedures were also used for this part of the study.

Table 5.1 Samples collected weekly by each abattoir

	Number of samples gathered		
	Abattoir A	**Abattoir B**	**Abattoir C**
Week 1	16	10	30
Week 2	12	27	NS*
Week 3	30	30	NS
Week 4	30	NS	30
Week 5	30	30	30
Total	118	97	90

*Samples could not be gathered

5.2.3 Sample preparation (first and second study)

Sample suspensions were prepared by homogenising collected samples (10 g) with peptone water (1:10 dilution m/v) for 30 sec using a stomacher (InterScience). After homogenisation a 1:10 dilution (v/v) was made with the Bolton broth (CM0983 - Oxoid, Basingstoke, United Kingdom) and *Arcobacter* broth (CM0965 - Oxoid, Basingstoke, United Kingdom) with 1 ml of the sample suspensions and processed as indicated below. For the second study, sample suspensions were also used for Polymerase Chain Reaction (PCR), as well as the detection of indicator microorganisms (Figure 5.2).

STUDY 1

STUDY 2

| Post-skinning | Post-evisceration | Post-chilling |

Post-evisceration (before final chilling)

30 samples collected at each slaughter point (n = 90 samples)

Samples collected weekly (3 abattoirs)

Sample suspension were made for each sample

Enumeration of indicator microorganism for each sample

Selective enrichment and streaked on appropriate agar

Sample suspensions pooled for molecular (PCR) analysis

Phenotypic confirmation

DNA extraction

Confirmation with PCR

Detection with PCR

Figure 5.2 Flow chart for the methodology followed for the first and second study

5.2.4 Cultural isolation of *Campylobacter* and *Arcobacter* species (first and second study)

After inoculation into Bolton broth (1:10 v/v) supplemented with the corresponding supplement (which contained 10 mg of cefoperazone, 10 mg of vancomycin, 10 mg of trimethoprim and 25 mg of cycloheximide) (SR0183E - Oxoid, Basingstoke, United Kingdom) and 10% horse blood (Medical Research Council, Delft, South Africa), the sample suspension was vortexed. The sample suspension was incubated at 37⁰C for 4-6 h at 42⁰C for 44-48 h, for the purpose of resuscitation and enrichment, respectively. Both resuscitation and enrichment occurred under micro-aerophilic conditions achieved by CampyGen sachets (CN00026 - Oxoid, Basingstoke, United Kingdom). After enrichment, a loopful (10 µl) of the enrichment was streaked onto Tryptose Blood Agar (TBA) (CM0233- Oxoid, Basingstoke, United Kingdom) and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (CM0739 - Oxoid, Basingstoke, United Kingdom) supplemented with the corresponding supplement (SR0155E - Oxoid, Basingstoke, United Kingdom) which contained cefoperazone (16 mg) and amphotericin B (5 mg).

After inoculation into *Arcobacter* broth (1:10 w/v) supplemented with the corresponding supplement (SR01745E - Oxoid, Basingstoke, United Kingdom) which contained cefoperazone (4 mg), teicoplanin (2 mg) and amphotericin B (5 mg), sample suspensions were vortexed and then incubated aerobically at 30⁰C for 48 h. After incubation, a loopful (10 µl) of the enrichment was streaked onto mCCDA (CM0739 - Oxoid, Basingstoke, United Kingdom) supplemented with the corresponding supplement (SR01745E - Oxoid, Basingstoke, United Kingdom). Plates were incubated at 30⁰C under aerobic conditions for 48 h (Merga *et al.*, 2013).

Confirmation of Campylobacter and Arcobacter species (first and second study)

After incubation, plates were examined for suspect colonies characterised as being small, round, convex, translucent, whitish/greyish colonies. Suspect *Campylobacter* and *Arcobacter* colonies (four colonies per sample) were streaked onto Columbia blood agar (CBA) (CM0331 - Oxoid, Basingstoke, United Kingdom) enriched with 5% horse blood (Medical Research Council, Delft, South Africa). Typical *Campylobacter* (incubated for 48h at 42°C) and *Arcobacter* (incubated for 48h at 30°C) colonies were tested for genus specific phenotypical and biochemical characteristics such as gram's staining, oxidase, catalase and growth at different temperature/atmosphere combinations. Briefly, pure colonies grown on CBA were suspended on a glass slide and fixed with heat and stained using a commercially available kit (Sigma-Aldrich, St. Louis, United States of America); stained slides were inspected under a microscope, where a pink stain was considered a positive reaction. Oxidase activity was determined by spreading a pure colony on commercially available oxidase strips (Merck, Darmstadt, Germany); the appearance of violet or deep blue colour within 10 sec was seen as a positive reaction. Catalase activity was determined through mixing a pure colony with one drop of hydrogen peroxide solution (3%); a rapid formation of bubbles was considered a positive reaction. Additionally, growth studies were conducted where presumptive *Campylobacter* spp. colonies were streaked on two CBA plates, with the first plate being incubated at 25°C under a micro-aerobic atmosphere for 40 h to 48 h and the second plate incubated at 42°C aerobic conditions for 40 h to 48 h; colony growth under these conditions was regarded as a negative reaction. Similarly, presumptive *Arcobacter* spp. colonies were streaked on CBA plates and placed

under a micro-aerophilic atmosphere at 42°C; growth under these conditions was regarded as a negative reaction.

Confirmed colonies were stored in Microbank™ vials (Davies Diagnostics, Johannesburg, South Africa) with 25 beads (3 mm) with a cryoprotectant liquid (1 ml) containing 10 -15% glycerol. Aseptic inoculation of Microbank™ vials was according to manufacturer's instructions. Briefly, an inoculating plastic loop was used to pick pure colonies and transferred to Microbank™ vials with cryoprotectant liquid, vials were closed tightly and inverted five times to emulsify the microorganism. In order to allow the microorganism to bind with the beads, the Microbank™ vials were left to stand for 2 min without disturbance, thereafter the cryoprotectant liquid was removed and the Microbank™ vials with isolates were stored at -80ºC until molecular confirmation of isolates could commence.

Molecular confirmation of Campylobacter and Arcobacter species (first and second study)

Previously preserved colonies were streaked onto CBA (CM0331 - Oxoid, Basingstoke, United Kingdom) and single colonies were suspended in 300 lysis buffer (10 mM Tris-HCl, 1% Triton X-100, 0.5 % TWEEN 20 and 1 mM EDTA) and then boiled (100ºC) for 10 min. After boiling the bacterial suspension was centrifuged at 5500 x g for 2 min and 250 µl of supernatant was transferred to new Eppendorf tubes with ice cold 99.9% ethanol. Thereafter, the equal parts supernatant and 99.9% ethanol were centrifuged at 13 000 x g for 2 min, the supernatant was discarded, and the pellet was left to dry at room temperature for $1 – 2$ h. After drying, 100 µl of 10 mM Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA) was added to the pellet and mixed by vortexing; extracted DNA could be used for PCR amplification using the primer set depicted in Table 5.2. The PCR reaction mixture (25 µl) contained 12.5 µl 2x *Taq* master-mix (Inqaba Biotec, South Africa) and 0.5 µl of each primer (10 p/mol).

PCR mixtures were subjected to the conditions depicted in Table 5.2 and reactions were performed in a Bio-Rad T100 Thermal Cycler (Bio-Rad, South Africa). A 1.5% agarose gel stained with EZ vision was used for the gel electrophoresis of the PCR products (10 µl). All gels ran for 90 min at 70 V. A 100 bp DNA ladder was used for each run, in order to size the products. A Bio-Rad Gel Doc XR+ System (Bio-Rad, South Africa) and Image Lab Software (version 5.2.1) was used for gel visualisation.

Table 5.2 Primer sequences and PCR conditions for the detection of *C. jejuni, C. coli, A. butzleri, A. skirrowii* and *A. cryaerophilus*

Identification	Sequence (5' to 3')	PCR conditions
Species specific primers (Negahdari *et al.*, 2016)		
C. jejuni	CTATTTTATTTTTGAGTGCTTGTG	one cycle of 2 min at 94ºC, 30
	GCTTTATTTGCCATTTGTTTTATTA	cycles each consisting of 40 sec at
		94ºC, 40 sec at 54ºC, 5 min at 72°C
C. coli	AATTGAAAATTGCTCCAACTATG	and a final extension step of 5 min
	TGATTTTATTATTTGTAGCAGCG	at 72ºC
Species specific primers (Soma, 2016)		
A. butzleri	CCTGGACTTGACATAGTAAGAATGA	one cycle of 5 min at 94ºC, 30
	CGTATTCACCGTAGCATAGC	cycles each consisting of 30 sec at
		94ºC, 1 min at 51ºC, 1 min at 72°C
A. skirrowii	GGCGATTTACTGGAACACA	and a final extension step of 10 min
	CGTATTCACCGTAGCATAGC	at 72ºC
A. cryaerophilus	TGCTGGAGCGGATAGAAGTA	
	AACAACCTACGTCCTTCGAC	

Molecular detection of Campylobacter **and** *Arcobacter species (study 1 and 2)*

In order to determine the presence of *Campylobacter* and *Arcobacter* species using PCR, the remaining sample suspension was incubated at 37ºC for 24 h under micro-aerophilic conditions. After incubation, the enrichment was used for DNA extraction; where applicable five 500 µl increments from sample suspensions were pooled. To extract DNA the ZymoBIOMICS DNA Miniprep Kit (Inqaba Biotec, South Africa) was used; DNA extractions were performed according to the manufacturer's instructions. Briefly, 250 µl of pooled enrichments was added to tubes with bashing beads and 750 µl lysis solution and processed with a bead beater for 5 min, after which, the tubes were centrifuged (10 000 x g for 1 min). The resultant supernatant was transferred to a Zymo-spin™ III-F filter in a collection tube and centrifuged (8000 x g for 1 min). To the filtrate collected in the collection tube, 1.2 ml of DNA binding buffer was added and the mixture properly mixed. After which, 800 µl of the mixture was transferred to a Zymo-spin™ IIC-Z column with a collection tube and centrifuged (10 000 x g for 1 min), this step was repeated twice. The Zymo-spin™ IIC-Z column was transferred to a new collection tube and 400 µl DNA wash buffer one was added and centrifuged (10 000 x g for 1 min), the filtrate was discarded. Then, 700 µl of DNA wash buffer two was added to Zymo-spin™ IIC-Z column in a collection tube and centrifuged (10 000 x g for 1 min), the filtrate being discarded. The previous step was repeated but 200 µl of DNA wash buffer two was added. The Zymo-spin™ IIC-Z column was transferred to a micro-centrifuge tube and 100 µl of DNase/RNase was added to the column, and left to incubate for 1 min, and centrifuged (10 000 x g for 1 min). The eluted DNA was transferred to a Zymo-spin™ III-HRC filter in a microcentrifuge tube and centrifuged (16 000 x g for 3 min). After filtration, eluted DNA could be used for PCR amplification using the primer sets depicted in Table 5.2.

PCR was performed in 25 µl volumes consisting of 12.5 µl 2x Taq master-mix (Inqaba Biotec, South Africa) and 0.5 µl of each primer (10 p/mol). All reagents used for the PCR reaction mixture were purchased from Inqaba Biotec, South Africa. PCR mixtures were subjected to the conditions depicted in Table 5.2. PCR, gel electrophoresis and gel visualisation were performed as indicated above.

5.2.5 Quality control

At each incubation point, control strains: *C. jejuni* subsp. *jejuni* ATCC 29428 (Davies Diagnostics, Johannesburg, South Africa), *C. coli, A. butzleri*, *A. skirrowii* and *A. cryaerophilus* were grown and streaked onto the appropriate agar and incubated under the conditions depicted for ISO 10272-1:2006, selective *Arcobacter* spp. method and the Cape Town protocol. Furthermore, a sterility test was conducted on all prepared plates by incubating plates for 24 h at 37°C, any growth on the plates was taken as a sign of contamination, and these plates were then discarded. Control strains were used as positive controls for the molecular confirmation and detection of *Arcobacter* spp. and *Campylobacter* spp.

5.2.6 Enumeration of microorganisms (second study)

Coliforms

Serial dilutions were prepared and transferred to marked petri dishes, and prepared Violet red bile agar (VRBG) (C23.500 - Merck, Darmstadt, Germany) was mixed with the corresponding dilution. Plates were left to solidify at room temperature before a second layer of VRBG was poured and left to solidify. Pour plates were then incubated at 37°C for 24 h. All dark pink to red colonies were counted manually.

E. coli

A serial dilution for the enumeration of *E. coli* was first prepared and transferred to marked petri dishes then Brilliance *E. coli*/coliform selective agar (CM1046 - Oxoid, Basingstoke, United Kingdom) was poured into plates and mixed with corresponding dilution. Plates were left to solidify, after which they were incubated at 37°C for 24 h. After incubation, purple colonies were counted manually.

5.3 Statistical analysis

For the first study, the presence of *Campylobacter* spp. and *Arcobacter* spp. was determined for all samples. Differences in the prevalence of *Arcobacter* spp. and *Campylobacter* spp. between three slaughter stages namely, post-skinning, post-evisceration and post-chilling was determined; with this data, graphs were configured to represent progression of contamination along the slaughter process. For the second study, coliform and *E. coli* counts were converted to logarithmic form. To show contamination for Abattoir A, B and C, $\log_{10}$ means for coliform and *E. coli* counts were determined, for each week of sampling. Furthermore, prevalence levels of *Campylobacter* spp. and *Arcobacter* spp. for Abattoir A, B and C, were determined for each week.

5.4 Results

5.4.1 Distribution of *Campylobacter* and *Arcobacter* species during slaughter (first study)

Selective methods were used for the detection of *Campylobacter* spp. and *Arcobacter* in ostrich meat samples. As seen in Figure 5.3, overall prevalence levels of 47% (42/90) and 80% (72/90) for *Campylobacter* spp. and *Arcobacter* spp. were determined for ostrich samples, respectively.
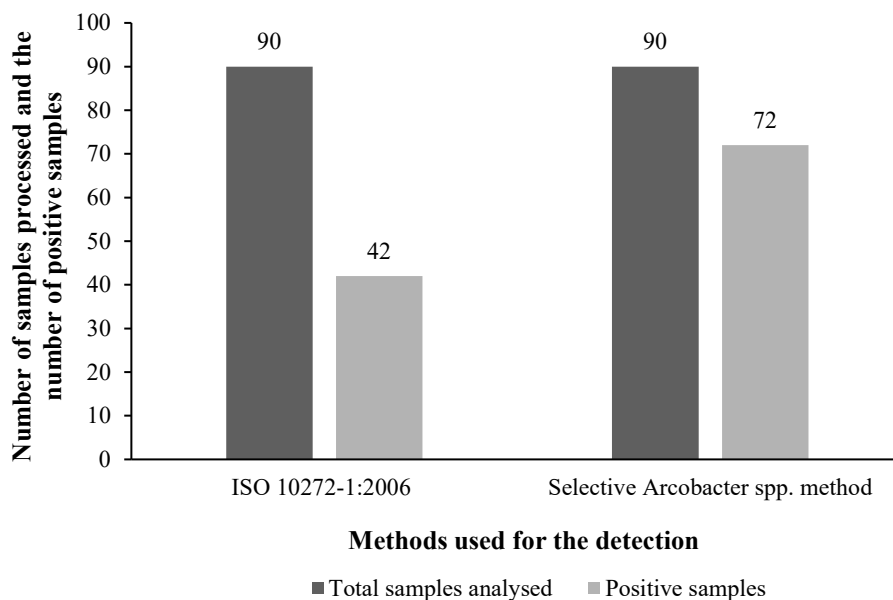
Figure 5.3 Total number of *Campylobacter* spp. and *Arcobacter* spp. positive samples as determined by selective methods

When the distribution of *Campylobacter* spp. (*C. jejuni*; Table 5.3) along the slaughter process was evaluated, *Campylobacter* spp. were detected after evisceration (post-evisceration; stage 2) and at post-chilling (stage 3). *Campylobacter* spp. were not detected in samples obtained post-skinning (stage 1) as seen in Figure 5.4. A *Campylobacter* spp. prevalence of 73% (22/30) was determined for samples obtained post-evisceration, whilst a slight reduction in prevalence was noted between evisceration and chilling, as 66% (20/30) of the samples gathered were positive for *Campylobacter* spp.

Unlike *Campylobacter* spp., *Arcobacter* spp. (*A. cryaerophilus*; Table 5.3) was isolated from all three stages of slaughter (Fig. 5.5). More specifically, at post-skinning, post-evisceration and post-chilling prevalence levels of 73% (22/30), 83% (25/30) and 67% (20/30), respectively were determined; the highest prevalence was exhibited at post-evisceration (Figure 5.5). Additionally, a slight decrease in prevalence between samples collected at post-evisceration and post-chilling was noted.
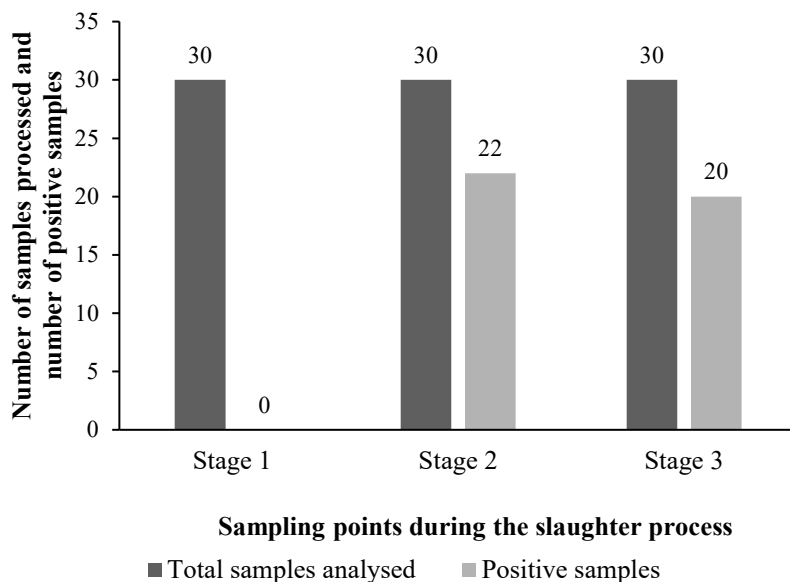
Figure 5.4 Occurrence of *Campylobacter* spp. on ostrich carcasses at three slaughter points, namely post-skinning (stage 1), post-evisceration (stage 2) and post-chill (stage 3)
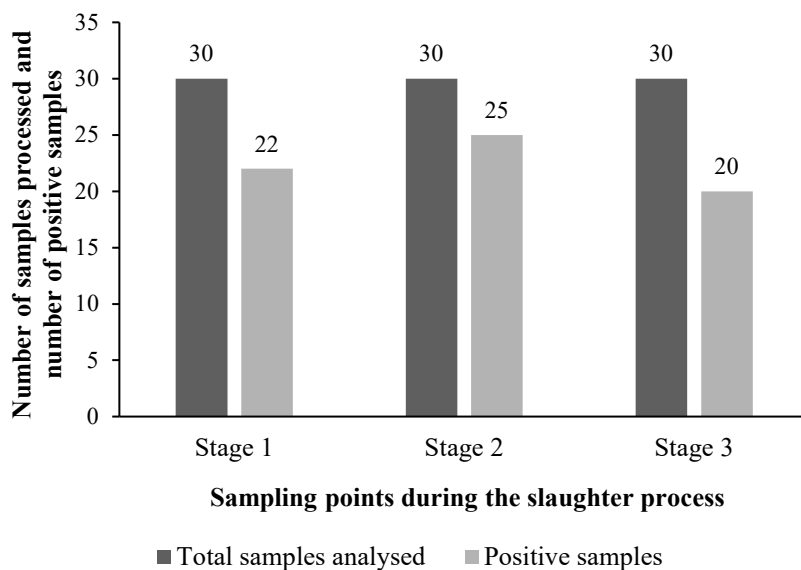
Figure 5.5 Occurrence of *Arcobacter* spp. on ostrich carcasses at three slaughter points, namely post-skinning (stage 1), post-evisceration (stage 2) and post-chill (stage 3)

Table 5.3 *Campylobacter* and *Arcobacter* species present at each slaughter stage as confirmed by PCR

	C. jejuni	*C. coli*	*A. butzleri*	*A. skirrowii*	*A. cryaerophilus*
Post-skinning	-	-	-	-	✓
Post-evisceration	✓	-	-	-	✓
Post-chilling	✓	-	-	-	✓

91

5.4.2 Prevalence of *Campylobacter* and *Arcobacter* spp. during slaughter (second study)

At the end of the sampling period (five weeks), 305 samples were collected from three ostrich abattoirs situated in the Western Cape region. Overall, using cultural and molecular methods, *Campylobacter* spp. and *Arcobacter* spp. was detected in 0 – 0.98% and 1.31 – 1.64% of the samples, respectively (Table 5.4). From week 1 to 4, *Campylobacter* spp. and *Arcobacter* spp. were not detected by cultural and molecular methods (Table 5.5). *Campylobacter* spp. and *Arcobacter* spp. were only seen on the 5th week of sampling from Abattoir A and C whilst *Arcobacter* spp. were detected in samples obtained from Abattoir B and C.

In ostriches, faecal contamination can be indicated by the presence of coliforms and *E. coli*. Average coliform counts of 0.35 $\log_{10}$ cfu/g, 1.02 $\log_{10}$ cfu/g and 0.23 $\log_{10}$ cfu/g were determined for Abattoir A, B and C, respectively (Table 5.4). An average *E. coli* count of 0.08 $\log_{10}$ cfu/g, 0.20 $\log_{10}$ cfu/g and 0.12 $\log_{10}$ cfu/g were determined for abattoir A, B and C, respectively (Table 5.4). From week 1 to 5, fairly low coliforms and *E. coli* counts were determined for all abattoirs (Table 5.5).

When faecal contamination (as represented by coliforms and *E. coli*) was explored in relation to the presence of species from the *Campylobacteraceae* family, a relationship could not be conclusively determined as, when faecal contamination was detected, *Campylobacter* and *Arcobacter* species were not detected by cultural methods and molecular methods (Table 5.5).

Table 5.4 Overall bacterial counts (coliforms and *E. coli*) and prevalence of species belonging to the *Campylobacteraceae* family

		Abattoirs			Mean/ overall prevalence
		A	**B**	**C**	
Microorganisms tested	Coliforms ($\log_{10}$ cfu/g)	0.35	1.02	0.23	0.53
	E. coli ($\log_{10}$ cfu/g)	0.08	0.20	0.12	0.13
	Campylobacter spp.	1/118 (0.85%)	0/97 (0%)	2/90 (2.22%)	3/305 (0.98%)
	PCR - *Campylobacter* spp.	0/24 (0%)	0/19 (0%)	0/18 (0%)	0/61 (0%)
	Arcobacter spp.	0/118 (0%)	1/97 (1.03%)	3/90 (3.33%)	4/305 (1.31%)
	PCR – *Arcobacter* spp.	0/24 (0%)	1/19 (5.26%)	0/18 (0%)	1/61 (1.64%)

Table 5.5 Bacterial counts (coliforms and *E. coli*) and prevalence of species belonging to the *Campylobacteraceae* family per abattoir per week

		Sampling times														
		Week 1			**Week 2**			**Week 3**			**Week 4**			**Week 5**		
		A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
		Cultural detection														
	Coliforms ($\log_{10}$cfu/g)	0	0.79	0	0.08	0.34	NS*	0.82	0.74	NS	0.81	NS	0.70	0.04	2.22	0
	E. coli ($\log_{10}$cfu/g)	0	0	0	0	0	NS	0.19	0.16	NS	0.23	NS	0.35	0	0.15	0
Microorganisms tested	*Campylobacter* spp.	0/16	0/10	0/30	0/12	0/27	NS	0/30	0/30	NS	0/30	NS	0/30	1/30	0/30	2/30
	Arcobacter spp.	0/16	0/10	0/30	0/12	0/27	NS	0/30	0/30	NS	0/30	NS	0/30	0/30	1/30	3/30
		Molecular detection (PCR)														
	Campylobacter spp.	0/3	0/2	0/6	0/3	0/5	NS	0/6	0/6	NS	0/6	NS	0/6	0/6	0/6	0/6
	Arcobacter spp.	0/3	0/2	0/6	0/3	0/5	NS	0/6	0/6	NS	0/6	NS	0/6	0/6	0/6	1/6

*Samples could not be gathered

5.5 Discussion

5.5.1 Distribution of *Campylobacter* and *Arcobacter* species during slaughter (first study)

During the slaughter of food-producing animals, bacterial contamination of carcasses can occur (Gouws *et al.*, 2017). As reviewed by Shange *et al.* (2019), slaughter steps such as defeathering, skinning and evisceration can contribute or aid in the contamination of carcasses with species belonging to the *Campylobacteraceae* family but on the other hand, contamination can also be reduced by process steps such as scalding, washing and chilling of carcasses. Evidence of contamination of carcasses with species belonging to the *Campylobacteraceae* family has been investigated during the slaughter of various food-producing animals such as chicken, cattle and pigs (Son *et al.*, 2007; De smet *et al.*, 2010; Maramski, 2012). As it stands *Campylobacter* spp. and *Arcobacter* spp. contamination in relation to ostrich carcasses is lacking. Therefore, for this part of the study, the opportunity of contamination was explored by determining the presence of *Campylobacter* spp. and *Arcobacter* spp. on ostrich carcasses during the slaughter process.

This study revealed that *Campylobacter* and *Arcobacter* species could be transferred to ostrich carcasses during slaughter, as overall, 47% and 80% of samples gathered were contaminated with *Campylobacter* spp. and *Arcobacter* spp., respectively (Fig. 5.3). At the first stage of sampling (post-skinning) *Campylobacter* spp. was not detected, whilst *Arcobacter* spp. was detected after skinning (Fig 5.4 and 5.5). Feathers and skin can harbour a number of microorganisms, including enteric pathogens such as *Arcobacter* spp. The presence of *Arcobacter* species has been proven on rabbit skin, poultry feathers and the skin of bovine carcasses (Lehner *et al.*, 2005; De Smet *et al.*, 2010; Šilha *et al.*, 2015). Furthermore, it has been shown that the transportation of chickens can lead to stress which can induce faecal excretion, resulting in faecal matter being present on feathers (Whyte *et al.*, 2001). However, it should be noted that ostrich farming practices tend to deter excessive dirtying of feathers with faecal matter, as feathers are an important commodity (Karama *et al.*, 2003). Nonetheless, for this study, skinning contributed to the contamination of ostrich carcasses. Even though the slaughter of ostriches in relation to species belonging to the *Campylobacteraceae* has not been extensively investigated, previous researchers have noted that the slaughter of ostriches could be comparable to the slaughter of bovine carcasses, as with the exception of the defeathering step, the skin is also not retained (Karama *et al.*, 2003). Therefore, findings from the study are in corroboration with results reported by De Smet *et al.* (2010), who found a significant increase in the prevelance of *Arcobacter butzleri* after skinning. During skinning, microorganisms present on the skin can be transferred to carcass surfaces directly or indirectly. Direct contamination can include the skin coming into contact with carcass surfaces and indirect contamination is the transfer of bacteria to carcass surfaces by personnel (hands) and equipment (De smet *et al.*, 2010). Additionally, it should be noted that skinning requires a great deal of effort and carcasses undergo a considerable amount of movement; this action can result in the creation of dust particles, which can land on the surface of carcasses, resulting in further contamination (De smet *et al*., 2010). The skinning of ostrich thighs is said to be a delicate operation that is executed with a certain level of difficulty (Karama *et al.,* 2003). Therefore, this study further provides evidence that the skinning step can indeed contribute in the increase of contamination levels found on

ostrich carcasses. It should also be noted that the ostrich skin itself is another important commodity for ostrich abattoirs therefore, it could be postulated that the care taken to preserve the state the of the skin might be antagonistic to the microbial quality of ostrich carcasses (ostrich meat), as previously reported by Karama *et al.* (2003).

An increase of *Campylobacter* spp. and *Arcobacter* spp. levels were seen after evisceration (Fig. 4 and 5). For this study, the evisceration step had the highest prevalence of *Campylobacter* and *Arcobacter* species, overall. This trend is in agreement with Figueroa *et al.* (2009), Khoshbakht *et al.* (2014), Reich *et al.* (2008), Maramski (2012) and De smet *et al.* (2010) during the slaughter of broiler chickens, pigs and beef carcasses. For the slaughter of most food-producing animals, the presence of species belonging to the *Campylobacteraceae* family on carcasses has been attributed to the evisceration step, as the puncture of viscera and subsequent faecal contamination can occur quite often, if this step is executed in an improper manner. As mentioned before, the slaughter of ostriches in relation to the contamination of carcasses with species belonging to the *Campylobacteraceae* family has not been explored extensively. However, faecal contamination has been implicated in the increase of faecal indicators (*E. coli*) on ostrich carcasses. Therefore, the increase in faecal indicators (*E. coli*) reported by Karama *et al.* (2003) and the increase in *Campylobacter* and *Arcobacter* species seen for the the evisceration step, can possibly be attributed to faecal contamination due to the perforation of the viscera or the leakage of intestinal fluid during evisceration (De smet, *et al.*, 2010; Van Driessche & Houf, 2007).

Ostrich carcasses were also sampled after chilling. The ostrich slaughter process requires the chilling of ostrich carcasses for 0-4°C for 24 h (Hoffman *et al.*, 2010). For this study, the chilling of carcasses resulted in a reduction of *Campylobacter* and *Arcobacter* species (Fig. 4 and 5). Previous research has also indicated that steps such chilling can reduce bacterial growth on carcasses. For instance, Grau (1988) reported a 50 to 100 fold reduction in *Campylobacter* spp. levels on calf and cattle carcasses after chilling. Also, during the slaughter of pigs, Maramski (2012) determined that carcasses (n = 7) previously contaminated with *Campylobacter* spp. were no longer contaminated after chilling, as *Campylobacter* spp. was detected in only one carcass. Similarly, De Smet *et al.* (2010) reported a reduction in *Arcobacter* spp. contaminated bovine carcasses after forced air cooling (24 h). The reduction in species belonging to *Campylobacteraceae* species seen in this study and previous research could be attributed to chemical changes in the lipid bilayer of microbial cells induced by low temperatures, which can result in a permanent physical damage of microbial cells (De Smet *et al.*, 2010). The results from this study are in agreement with the notion that contamination levels of species belonging to the *Campylobacteraceae* family can be reduced through chilling. However, it should be noted that species were not completely eradicated as over 50% of carcasses (Fig. 5.4 and 5.5) were still contaminated. The authors urge further investigation of chilling efforts within this abattoir, as factors such as spacing between carcasses could potentially curb the efficiency of this process step. Alternatively, this particular abattoir should investigate the use of other chilling techniques, such forced air cooling. During the slaughter of bovine carcasses, De Smet *et al.* (2010) detected an *Arcobacter* spp. prevalence of 37.4% and after forced air cooling (24 h), a significant reduction was seen as *Arcobacter* spp. was detected in only 7.4% of the bovine carcasses.

5.5.2 *Campylobacter* and *Arcobacter* spp. during slaughter (second study)

In the second study, an attempt was made to determine the occurrence of *Campylobacter* spp. and *Arcobacter* spp. to see whether abattoirs should be required to test samples on a regular basis. Therefore, samples were collected from three abattoirs on a weekly basis before chilling as mandated by VPN 52/2018. This study was also used to see if faecal contamination as indicated by faecal indicators (*E. coli* and coliforms), could be used to predict the presence of species that belong to the *Campylobacteraceae* family. It should be noted that the second study differs from the first study, as random sampling was conducted, whilst for the first study, the flock sampled was known to be infected with *Campylobacter* spp. and *Arcobacter* spp.

From this trial, the occurrence of *Campylobacter* spp. was low, as weekly results showed that *Campylobacter* spp. never exceeded a prevalence level of 6% (Table 5.5). Even though microbiological specifications for ostriches in relation to species that belong to *Campylobacteraceae* family are not set for ostrich carcasses, they are set for the slaughter of chickens and pigs. For instance, poultry and pork carcasses are considered unsafe for human consumption when *C. jejuni* and *C. coli* are found in 25% (5/25 carcasses) and 9.63% (1.92/20 carcasses) of poultry and pork carcasses tested, respectively. By these standards, results from this study show that ostrich carcasses would still be safe for consumption, as results were considerably low. The highest occurrence for *Arcobacter* spp. was seen in the 5th week of sampling for Abattoir C, however comparisons to microbial specifications cannot be made as guidelines have not yet been set (for the slaughter of all food-producing animals). Furthermore, from this trial it was also seen that faecal contamination as indicated by the presence of *E. coli* and coliforms was low throughout the complete sampling period. These results could potentially indicate that during the time of sampling, the slaughter process was conducted in a hygienic manner and if faecal contamination did occur (see study 1) it was controlled or mitigated during slaughter (Sampers *et al.*, 2010). The low *E. coli* and coliforms counts seen for this study can also be attributed to the fact that sampling was conducted when slaughter volumes were low for all abattoirs (A. Olivier, 2019, Doctor of Veterinary medicine, South African Ostrich Business Chamber, personal communication, 30 September). Other factors could be that during the time of sampling the flocks slaughtered were not infected with *Campylobacter* spp. and *Arcobacter* spp. As seen in Chapter 4, it is possible to obtain flocks that are free from these species or are low in prevalence.

Considerable effort to study *Campylobacter* spp. (in particular) in relation to faecal indicators such as *E. coli* and coliforms has been made (Berrang & Dickens, 2000). This research effort can be attributed to the fact that enumeration techniques for generic *E. coli* are easier and also cheaper (Habib *et al.*, 2012). Research on broiler chicken carcasses has shown that *E. coli* can exhibit the same trend as *Campylobacter* spp. during slaughter i.e. increase after evisceration and decrease after chilling (Pacholewicz *et al.*, 2016). Other researchers have noted that presence of *E. coli* could be an indication of the presence of *Campylobacter* species (Duffy *et al.*, 2014). However, most of this research (if not all) is conducted on broiler carcasses. In broiler chickens *E. coli* and *Campylobacter* spp. are present at high levels (>3 $\log_{10}$ cfu/g) and therefore a correlation/relationship can be clearly seen (Roccato *et al.*, 2018b). Unlike the present

study, where all microorganisms were present at extremely low levels. Therefore, due to a low presence of faecal indicators, *Campylobacter* spp. and *Arcobacter* spp., a relationship could not be conclusively proven.

5.6 Conclusion

Campylobacter and *Arcobacter* species are closely related pathogens that can affect public health. The most likely route that leads to infections in humans is the consumption of contaminated meat and meat products; this contamination can occur during slaughter. The first study showed that during skinning and evisceration, ostrich carcasses could be contaminated with species that belong to the *Campylobacteraceae* family. This study also showed that the chilling process can potentially reduce contamination. The second study demonstrated that routine testing of *Campylobacter* spp. and *Arcobacter* could yield low levels of *Campylobacter* spp., *Arcobacter* spp. and faecal contamination as represented by coliform and *E. coli* counts provided that good hygienic practices are strictly followed.

This chapter showed that contamination occurs; however, contamination is not only important concerning the presence of bacterial pathogens on carcasses, it can also be important with regards to the presence of antibiotic resistant bacterial pathogens on carcasses. With this mind, in Chapter 6, the antibiotic resistance of *Campylobacter* spp. and *Arcobacter* spp. isolates gathered at primary production and during slaughter will be assessed.

5.7 References

Berrang, M.E. & Dickens, J.A. (2000). Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. *Journal of Applied Poultry Research*, **9**, 43–47.

Collado, L. & Figueras, M.J. (2011). Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clinical Microbiology Reviews*, **24**, 174–192.

De Smet, S., Zutter, L. De, Hende, J. Van & Houf, K. (2010). *Arcobacter* contamination on pre- and post-chilled bovine carcasses and in minced beef at retail. *Journal of Applied Microbiology*, **108**, 299–305.

Duffy, L.L., Blackall, P.J., Cobbold, R.N. & Fegan, N. (2014). Quantitative effects of in-line operations on *Campylobacter* and *Escherichia coli* through two Australian broiler processing plants. *International Journal of Food Microbiology*, **188**, 128–134.

Evers, E.G., van der Fels-Klerx, H.J., Nauta, M.J., Schijven J.F. & Havelaar, A.H. (2008). *Campylobacter* source attribution by exposure assessment. *International Journal of Risk Assessment and Management*, **8**, 174–189.

Figueroa, G., Troncoso, M., Ĺopez, C., Rivas, P. & Toro, M. (2009). Occurrence and enumeration of *Campylobacter* spp. during the processing of Chilean broilers. *BMC Microbiology*, **9**, 1–6.

Gill, C.O. & Harris, L.M. (1982). Contamination of red-meat carcasses by *Campylobacter fetus* subsp. jejuni. *Applied and Environmental Microbiology*, **43**, 977–980.

Gouws, P.A., Shange, N., & Hoffman, L.C. (2017). Microbial quality of springbok (*Antidorcas marsupialis*) meat in relation to harvesting and production process. In: *Game meat hygiene: Food safety and security* (edited by P. Paulsen, A, Bauer, & F.J.M. Smulders). Pp. 223-228. Netherlands: Wageningen Academic Publishers.

Grau, F.H. (1988). *Campylobacter* j*ejuni* and *Campylobacter* hyointestinalis in the intestinal tract and on the carcasses of calves and cattle. *Journal of Food Protection*, **51**, 857–861.

Habib, I., Zutter, L. De, Huffel, X. Van, Geeraerd, A.H. & Uyttendaele, M. (2012). Potential of *Escherichia coli* as a surrogate indicator for postchill broiler carcasses with high *Campylobacter* counts. *Food Control*, **25**, 96–100.

Hoffman, L.C. (2012). Advances in the electrical stunning and bleeding of ostriches. *Animal Welfare*, **21**, 9 – 13.

Hoffman, L.C., Britz, T.J. & Schnetler, D.C. (2010). Prevalent organisms on ostrich carcasses found in a commercial abattoir. *Journal of the South African Veterinary Association*, **81**, 151–155.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, **28**, 687–720.

Karama, M., Jesus, A.E. De & Veary, C.M. (2003). Microbial quality of ostrich carcasses produced at an export-approved South African abattoir. *Journal of Food Protection*, **66**, 878–881.

Keener, K.M., Bashor, M.P., Curtis, P.A., Sheldon, B.W. & Kathariou, S. (2004). Comprehensive review of *Campylobacter* and poultry processing. *Reviews in Food Science and Food Safety*, **3**, 105–116.

Khoshbakht, R., Tabatabaei, M., Shirzad Aski, H. & Seifi, S. (2014). Occurrence of *Arcobacter* in Iranian poultry and slaughterhouse samples implicates contamination by processing equipment and procedures. *British Poultry Science*, **55**, 732–736.

Lehner, A., Tasara, T. & Stephan, R. (2005). Relevant aspects of *Arcobacter* spp. as potential foodborne pathogen. *International Journal of Food Microbiology*, **102**, 127–135.

Maramski, A. (2012). Prevalence of *Campylobacter* spp. in Pig Slaughter Carcasses During the Processing. *Trakia Journal of Sciences*, **10**, 62–67.

Markets and Economic Research Centre (MERC), (2010). The South African Ostrich sub-sector. [Internet document]. URL http://www.namc.co.za/upload/all%20reports/Ostrich%20Value%20Chain%20Report.pdf 02/10/2016.

Merga, J.Y., Williams, N.J., Miller, W.G., Leatherbarrow, A.J.H., Bennett, M., Hall, N., Ashelford, K.E. & Winstanley, C. (2013). Exploring the diversity of *Arcobacter butzleri* from cattle in the UK using MLST and whole genome sequencing. *PLoS ONE*, **8**, 1–12.

Negahdari, B., Shirazi, M., Malekshahi, Z.V., Kadkhodazedeh, M., Hajikhani, S. & Rahmati, M. (2016). Identification of *Campylobacter jejuni* and *Campylobacter coli* from diarrheic samples Using PCR. *International Journal of Health Studies*, **2**, 1–3.

Pacholewicz, E., Swart, A. & Havelaar, A.H. (2016). Explanatory variables associated with *Campylobacter* and *Escherichia coli* concentrations on broiler chicken carcasses during processing in two slaughterhouses. *Journal of Food Protection*, **79**, 2038-2047.

Ramees, T.P., Dhama, K., Karthik, K., Rathore, R.S., Kumar, A., Saminathan, M., Tiwari, R., Malik, Y.S. & Singh, R.K. (2017). *Arcobacter*: An emerging food-borne zoonotic pathogen, its public health concerns and advances in diagnosis and control - A comprehensive review. *Veterinary Quarterly*, **37**, 136–161.

Reich, F., Atanassova, V., Haunhorst, E. & Klein, G. (2008). The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter*. *International Journal of Food Microbiology*, **127**, 116–120.

Robert, E., Levine, M.M., Lou, M., Hughes, T.P. & Martin, J. (1988). Experimental *Campylobacter jejuni* Infection in humans. *The Journal of Infectious Diseases*, **157**, 472–479.

Roccato, A., Mancin, M., Barco, L., Cibin, V., Antonello, K., Cocola, F. & Ricci, A. (2018). International Journal of Food Microbiology Usefulness of indicator bacteria as potential marker of *Campylobacter* contamination in broiler carcasses. *International Journal of Food Microbiology*, **276**, 63–70.

Samie, A., Obi, C.L., Barrett, L.J., Powell, S.M. & Guerrant, R.L. (2007). Prevalence of *Campylobacter* species, *Helicobacter pylori* and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: Studies using molecular diagnostic methods. *Journal of Infection*, **54**, 558–566.

Shange, N., Gouws, P. & Hoffman, L.C. (2019). *Campylobacter* and *Arcobacter* species in food - producing animals: prevalence at primary production and during slaughter. *World Journal of Microbiology and Biotechnology*, **35**, 146.

Van Driessche, E. & Houf, K. (2007). Characterization of the *Arcobacter* contamination on Belgian pork carcasses and raw retail pork. *International Journal of Food Microbiology*, **118**, 20–26.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N., Zissis, G. & Butzler, J. (2004). *Arcobacter* species in humans. *Emerging Infectious Diseases*, **10**, 1863-1867.

Veterinary Procedural Notices (VPN) 52. 2018. Guidelines on the verification of compliance with process hygiene control and microbiological food safety performance objectives under the Meat Safety Act. Available at http://www.rmaa.co.za/wp-content/uploads/2018/12/VPN-52-Microbiological-Reference-Criteria-for-Meat-19-October-2018-Draft.pdf. Accessed at 5 August 2019.

Whyte, P., Collins, J.D., McGill, K., Monahan, C. & O'Mahony, H. (2001). The effect of transportation stress on excretion rates of *Campylobacters* in market-age broilers. *Poultry Science*, **80**, 817–820.

CHAPTER 6

Antibiotic resistance patterns of *Campylobacter* spp. and *Arcobacter* spp. isolates obtained from ostriches and ostrich carcasses from the Western Cape, South Africa

Abstract

Antibiotic resistance patterns for 42 *Campylobacter* spp. and 42 *Arcobacter* spp. isolates collectively obtained from ostriches at primary production and during the slaughter of ostriches was determined. Antibiotic resistance was determined using the Kirby-Bauer disc diffusion method as depicted by the Clinical and Laboratory Standards Institute (CLSI) guidelines. *Campylobacter* spp. isolates were resistant to antibiotics in the following order: cephalothin and vancomycin (100%), erythromycin (90.48%), tetracycline (47.62%), nalidixic acid (11.90%) and ciprofloxacin (7.14%). The highest susceptibility for *Campylobacter* spp. was seen for nalidixic acid (88.10%) and ciprofloxacin (71.43%). *Arcobacter* spp. were resistant to antibiotics in the following order: cephalothin and vancomycin (100%), erythromycin (69.05%), tetracycline (45.24%), nalidixic acid (35.71%) and ciprofloxacin (21.43%). *Arcobacter* spp. isolates were also susceptible to ciprofloxacin and nalidixic acid. Overall, 59.52% and 61.90% of the *Campylobacter* spp. and *Arcobacter* spp. isolates, respectively showed resistance to antibiotics. Furthermore, most of the *Campylobacter* spp. (92.86%) and *Arcobacter* spp. (80.95%) isolates could be classified as multi-drug resistant, as they were resistant to three or more classes of antibiotics. Overall, results from this study showed substantial resistance in *Campylobacter* spp. and *Arcobacter* spp. isolated from ostriches and ostrich carcasses.

6.1 Introduction

Various food-producing animals such as cattle, sheep pigs and poultry (including ostriches as seen in Chapters 3 to 5) harbour species that belong to the *Campylobacteraceae* family, as reviewed by Shange *et al.* (2019). Poultry species are seen as the primary reservoir and by extension, contaminated poultry meat and poultry meat products are the main source of *Campylobacter* spp. and *Arcobacter* spp. related infections in humans (Shange *et al.*, 2019). The consumption of contaminated food typically causes gastrointestinal infections, characterised as a self-limiting gastroenteritis syndrome manifested as abdominal cramps, abdominal pain, nausea, fever and diarrhoea (Altekruse & Tollefson, 2003; Kaakoush *et al.*, 2015). For self-limiting infections treatment is usually not administered, however in some cases, especially when an infection occurs in immunocompromised individuals, the infection can be more severe and long lasting (Vandenberg *et al.*, 2004; Collado & Figueras, 2011); when this is the case, antibiotics are administered. Treatment compromises of antibiotics that fall within the following classes: macrolide, tetracycline, quinolone and aminoglycoside (Moore *et al.*, 2006); in this regard antibiotics are important for public health.

Antibiotics are also important for animal production as at primary production, antibiotics can be added to feed at sub-therapeutic levels to act as low cost growth promoters, which aid feed utilisation in farmed animals (Saad & Ahmed, 2018). Antibiotics can also be administered to food-producing animals at

therapeutic levels in order to treat diseases (Baynes *et al.*, 2016). Overall, the use of antibiotics ensures that farmers produce healthy food-producing animals and ensures productivity (Cogliani *et al.*, 2011; Braykov *et al.*, 2016). However, frequent use and improper use of antibiotics at primary production can create a selective pressure whereby, bacteria adapt and consequently give rise to a more resistant bacterial population (Gouws & Brozel, 2000; Olivier, 2014; van den Honert *et al.*, 2018). Antibiotic resistance in bacteria is defined as the rise of new mutations in the bacterial genome or the acquisition of genes that code for resistance, which ultimately change the defensive mechanisms of the bacteria. Mutation or acquisition can be aided or facilitated by the frequent use of antibiotics (Witte, 1998). The relationship between the use of antibiotics at primary production and antibiotic resistance in important foodborne pathogens is well recognised which is why countries such as Sweden, Denmark, the Netherlands, the United Kingdom and other European union countries have banned the non-therapeutic and prophylactic use of essential antibiotics at primary production (Cogliani *et al.*, 2011). However, in some countries, especially developing countries such as South Africa, antibiotics are still used at primary production for various food-producing animals (Zhang *et al.*, 2009; Williams-Nguyen *et al.*, 2016) including ostriches, as ostriches reared under the intensive feedlot system are typically served feed with growth promoters (Brand, 2014) and antibiotics are still administered to ostriches to treat diseases (Olivier, 2014).

Species that belong to the *Campylobacteraceae* family can be resistant to various antibiotics such as tetracycline, cephalosporin, quinolones, nalidixic acid, vancomycin, erythromycin and ciprofloxacin (Son *et al.*, 2007; Giacomelli *et al.*, 2014; Rahimi, 2014; Karikari *et al.*, 2017). The aforementioned antibiotics can have the same mode of action or fall within the same class as those used to treat *Campylobacter* spp. and *Arcobacter* spp. related infections in humans. Due to this cross-over, antibiotic resistance of species that belong to the *Campylobacteraceae* family can be a threat to public health. Furthermore, despite the export potential and consistent demand for ostrich meat (MERC, 2010), there is lack of research on antibiotic resistance from microorganisms obtained from ostriches and ostrich meat, in fact this is a fairly unknown field of study. Therefore, the aim of this study was to determine the antibiotic resistance patterns of *Campylobacter* and *Arcobacter* species isolated from ostrich carcasses and ostriches from South Africa.

6.2 Materials and method

6.2.1 Resuscitation of isolates

Campylobacter spp. and *Arcobacter* spp. isolates (Table 6.1) previously obtained from ostrich chicks (Chapter 3), live ostriches (Chapter 4) and from ostrich carcasses (Chapter 5) were resuscitated in order to test for antibiotic resistance. Following manufacturer's instructions, resuscitation occurred in the following manner: Microbank™ vials were aseptically opened and using a sterile plastic needle, at least one bead from each vial was removed and aseptically placed on the surface of Tryptose blood agar (TBA) plates (CMO233 - Oxoid, Basingstoke, United Kingdom) supplemented with 5% horse blood (Medical Research Council, Delft, South Africa) and streaked across the surface. It should be noted that Microbank™ vials were quickly returned to the freezer (-80ºC) in order to curb excessive temperature changes, which might

affect viability. Inoculated plates were stored under micro-aerophilic conditions achieved with CampyGen sachets (CN00026 - Oxoid, Basingstoke, United Kingdom) for 48 h at 37ºC and then checked for growth. Colonies were again sub-cultured on TBA and incubated under micro-aerophilic conditions for 24 h at 37ºC in order to obtain pure single colonies.

Table 6.1 Origin of *Campylobacter* spp. and *Arcobacter* spp. isolates used to determine antibiotic resistance

Isolates	Ostrich chicks and live ostriches	Ostrich carcasses	Total number of isolates
Campylobacter spp.	28	14	42
Arcobacter spp.	25	17	42

6.2.2 Antibiotic resistance testing

For this study, antibiotic resistance was determined for antibiotics (Table 6.2) associated with the treatment of *Campylobacter* spp. and *Arcobacter* spp. related infections in humans and/or usage at primary production as reported in other studies.

To determine antibiotic resistance of *Campylobacter* spp. and *Arcobacter* spp. isolates the Kirby-Bauer disc diffusion method was used as depicted by the Clinical and Laboratory Standards Institute (CLSI) guidelines (2016a). Bacterial suspensions for *Campylobacter* spp. and *Arcobacter* spp. isolates were prepared in the following manner: pure colonies were inoculated into autoclaved distilled water and mixed thoroughly by vortexing. Thereafter, the inoculum's turbidity was compared to the 0.5 McFarland standard (Lasec, South Africa). If turbidity was not in accordance to the 0.5 McFarland standard, autoclaved distilled water was added to the bacterial suspension to lessen the turbidity or more colonies were added to the bacterial suspension to increase the turbidity. Whenever adjustments were made, the bacterial suspension was vortexed again before comparing to the 0.5 McFarland standard.

After the preparation of the bacterial suspension, a sterile cotton swab (Lasec, South Africa) was immersed in the bacterial suspension, excess fluid was removed by pressing the immersed swab on the wall of the tube and the swab was uniformly streaked over the entire surface of prepared Mueller Hinton agar (MHA) plates (C105437 - Merck, Darmstadt, Germany) supplemented with 5% horse blood (Medical Research Council, Delft, South Africa). Each isolate was streaked in duplicate. MHA plates were left to dry at room temperature for to 3 to 5 min. Once dried, six antibiotic discs (Oxoid, Basingstoke, United Kingdom) were dispensed on the surface (Table 6.2) of MHA plates. After the antibiotic discs were dispensed, the MHA plates were incubated at 37ºC for 48 h under micro-aerophilic conditions achieved with a CampyGen sachet (CN00026 - Oxoid, Basingstoke, United Kingdom) (CLSI, 2016a).

For *Campylobacter* spp. and *Arcobacter* spp., diameter zones were measured in millimetres (mm) and interpreted as susceptible, intermediate or resistant for each antibiotic (Table 6.2). It should be noted that previous authors have expressed that there is lack of interpretative CLSI criteria for species that belong to the *Campylobacteraceae* family (Goni *et al.*, 2018). Therefore, as employed by previous researchers,

criteria set for the *Enterobacteriaceae* family was used as breakpoints for *Campylobacter* spp. and *Arcobacter* spp. (Goni *et al.*, 2018). Furthermore, in the case of erythromycin, cephalothin and vancomycin, breakpoints depicted for *Staphylococcus aureus*, *Streptococcus* and *Enterococcus* were used, respectively as CLSI *Campylobacter* spp., *Arcobacter* spp. and *Enterobacteriaceae* breakpoints for these antibiotics have not yet been established (Goni *et al.*, 2018).

Table 6.2 A list of antibiotics used to determine antibiotic resistance, the corresponding class, concentration and breakpoints (zone diameters) as set by the Clinical and Laboratory Standards Institute (2016b).

| Class	Antibiotic (code)	Concentration	Breakpoints (zone diameter (mm))		
			Susceptible (S)	Intermediate (I)	Resistant (R)
Quinolone	Ciprofloxacin (Cip)	5 µg	≥21	16-20	≤15
Macrolide	Erythromycin (Ery)	15 µg	≥23	14-22	≤13
Tetracycline	Tetracycline (T)	30 µg	≥15	12-14	≤11
Cephalosporin	Cephalothin (Ceph)	30 µg	≥24	-*	-
Fluoroquinolone	Nalidixic acid (NA)	30 µg	≥19	14-18	≤13
Glycopeptide	Vancomycin (V)	5 µg	≥17	15-16	≤14

*Criteria not set

6.3 Statistical analysis

To compare resistance levels for each antibiotic, diameter zones were classified as resistance, intermediate and susceptible. Then for each antibiotic the overall percentage of isolates that were resistant, intermediate and susceptible was calculated. This data was used for the construction of tables and graphs on Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

6.4 Results

Antibiotic resistance was determined for *Campylobacter* spp. and *Arcobacter* spp. isolates from ostriches and ostrich carcasses. Overall, from Figure 6.1, 59.52% and 61.90% of the *Campylobacter* spp. and *Arcobacter* spp. isolates, respectively showed resistance to antibiotics. Whilst, susceptibility was seen for 35.32% and 32.94% of the *Campylobacter* spp. and *Arcobacter* spp. isolates, respectively.

a   b
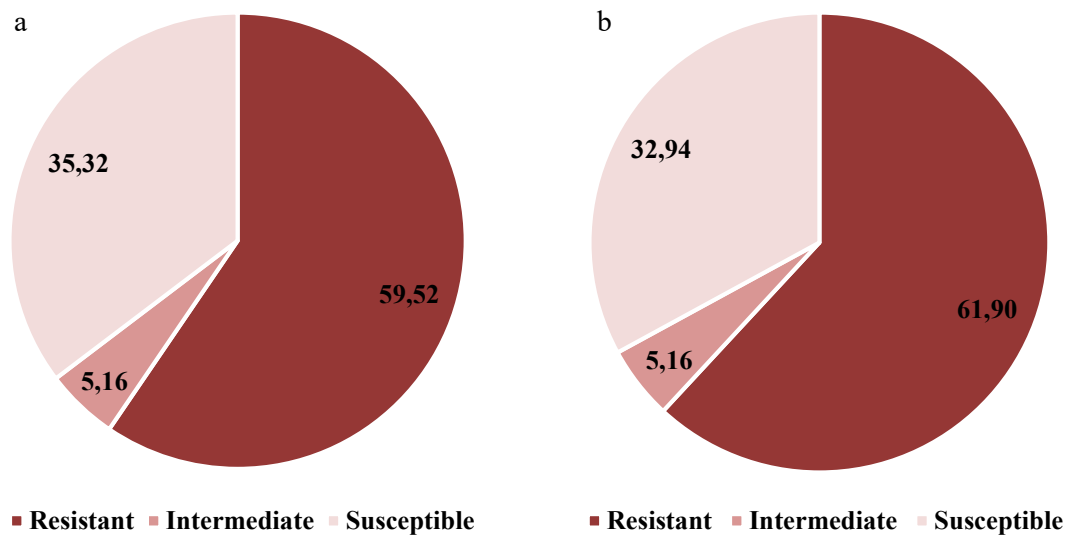
Figure 6.1 Overall antibiotic resistance levels (%) of *Campylobacter* spp. (a) and *Arcobacter* spp. (b) from ostriches and ostrich carcasses.

For this study *Campylobacter* spp. isolates were resistant in the following order (Table 6.3): cephalothin and vancomycin (100%), erythromycin (90.48%), tetracycline (47.62%), nalidixic acid (11.90%) and ciprofloxacin (7.14%). The highest susceptibility for *Campylobacte*r spp. was seen for nalidixic acid (88.10%) and ciprofloxacin (71.43%). A similar trend for *Arcobacter* spp. was witnessed as most of the isolates were resistant to antibiotics in the order of: cephalothin and vancomycin (100%), erythromycin (69.05%), tetracycline (45.24%), nalidixic acid (35.71%) and ciprofloxacin (21.43%). *Arcobacter* spp. isolates were mostly susceptible to ciprofloxacin (71.43%) and nalidixic acid (57.15%) (Table 6.3).

Table 6.3 Antibiotic resistance of *Campylobacter* spp. and *Arcobacter* spp. isolates

		Antibiotics					
		Cip	**Ery**	**T**	**Ceph**	**NA**	**V**
Campylobacter spp. (n = 42)	**R**	7.14%	90.48%	47.62%	100%	11.90%	100%
	I	21.43%	0%	0%	0%	0%	0%
	S	71.43%	9.52%	52.38%	0%	88.10%	0%
Arcobacter spp. (n = 42)	**R**	21.43%	69.05%	45.24%	100%	35.71%	100%
	I	7.14%	14.29%	2.38%	0%	7.14%	0%
	S	71.43%	16.66%	53.37%	0%	57.15%	0%

In this study antibiotic resistance was further classified as resistant (R; resistant to one class of antibiotics), extensively drug resistant (XDR; resistance to two different classes of antibiotics) and multi-drug resistant

104

(MDR; resistance to more than three different classes antibiotics) (Magiorakos *et al.*, 2011; King & Schmidt, 2017) (Fig. 6.2). Most *Campylobacter* spp. and *Arcobacter* spp. isolates were classified as multi-drug resistant, whilst none of the isolates were classified as being resistant to one class of antibiotics. For *Campylobacter* spp. most of the isolates (42.86%) were resistant to four classes of antibiotics namely, macrolides, tetracycline, cephalosporin and glycopeptides (Table 6.4). Most of the *Arcobacter* spp. isolates (23.81%) were resistant to three antibiotic classes namely, macrolides, cephalosporin and glycopeptides (Table 6.4).
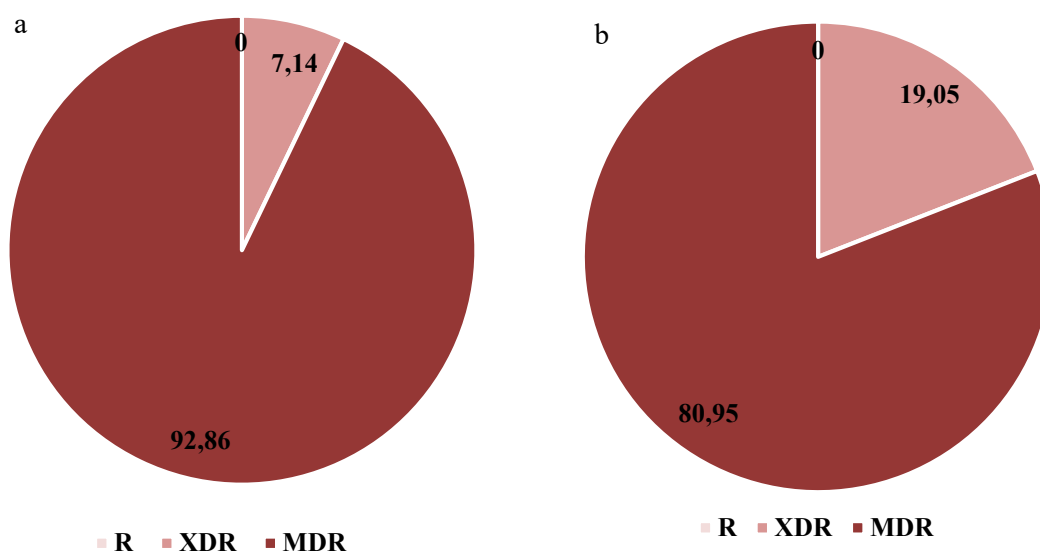
Figure 6.2 The overall classification (%) of *Campylobacter* spp. (a) and *Arcobacter* spp. (b) isolates as R (resistance to one different class of antibiotics), XDR (resistance to two different classes of antibiotics) and MDR (resistance to more than three different classes antibiotics`)

Table 6.4 Antibiotic resistant patterns for *Campylobacter* spp. and *Arcobacter* spp. isolates

Antibiotic resistant pattern	Antibiotic classes	Number of resistant isolates (%)	
		Campylobacter spp.	*Arcobacter* spp.
Resistant to all	5	2.38	16.67
Ceph-V	2	7.14	19.06
T-Ceph-V	3	-*	11.90
E-Ceph-V	3	42.86	23.81
E-T-Ceph-V	4	19.05	7.14
E-Ceph-NA-V	4	-	9.52
C-E-T-Ceph-V	4	-	2.38
C-Ceph-NA-V	3	-	2.38
E-T-Ceph-NA-V	5	21.43	7.14
C-E-Ceph-V	4	2.38	-
C-T-Ceph-V	4	4.76	-

*Combination not found

105

6.5 Discussion

In the ostrich industry (primary production), antibiotics can be used for the treatment of various diseases and or infections and can also be used as growth promoters (Brand, 2006; Olivier, 2014). However, it is stressed that usage should be controlled and supervised, as the overuse of antibiotics can encourage antibiotic resistant bacteria (Olivier, 2014) and can also encourage the spread of resistance genes from resistant bacteria to susceptible bacteria (Witte, 1998).

Cephalothin is considered to be one of the six therapeutically useful cephalosporins (Yamana & Tsuji, 1976; Vasoo *et al.*, 2014) and vancomycin can be used to treat infections related to species that belong to the *Campylobacteraceae* family (Arguello *et al.*, 2015). For this study, *Campylobacter* spp. and *Arcobacter* spp. isolates were highly resistant to cephalothin and vancomycin (Table 6.3). Previously, high resistance to cephalothin and vancomycin has been attributed to *Campylobacter* spp. and *Arcobacter* spp.'s intrinsic resilience towards cephalothin and vancomycin. In fact, the resistance to cephalothin is considered a useful tool used for the confirmation of species that belong to the *Campylobacteraceae* family (Yamana & Tsuji 1976; Lastovica, 2006) and vancomycin is typically included in selective media (Rahimi, 2014). For gram-negative bacteria the ability to resist vancomycin has been attributed to the inability of glycol-peptides to pass through porins, which govern the movement of hydrophilic molecules across the cell wall (Fanelli *et al.*, 2019). This notion was corroborated by this study as all *Campylobacter* spp. and *Arcobacter* spp. isolates were resistant to vancomycin. Similar results have been noted by Shirzad-Aski *et al.* (2016) and Soma *et al.* (2017). Additionally, it could be postulated that the cephalothin resistance determined in this study might have less to do with the practices at primary production and more to do with the physiology of *Campylobacter* and *Arcobacter* species, as the resistance towards this antibiotic was documented as early as 1986 in patients with *Campylobacter* enterocolitis (Sellu, 1986).

Previously, *Campylobacter* and *Arcobacter* species obtained from South African patients have shown that resistance towards erythromycin is increasing (Lastovica, 2006; Samie *et al.*, 2007; Simango, 2013). Nonetheless, erythromycin is still considered to be the first drug of choice when dealing with severe campylobacteriosis symptoms (Shobo *et al.*, 2016; Yoon *et al.*, 2017). For this study, erythromycin had the third highest resistance of 90.48% and 69.05% for *Campylobacter* spp. and *Arcobacter* spp., respectively (Table 6.3). The high levels of resistance could be an indication of overuse either to treat infections in ostriches or as a feed additive. Previous studies indicated that resistance of erythromycin was low (Acheson & Allos, 2002). However, the high resistance from this study could be an indication that the resistance of macrolides such as erythromycin is increasing. In fact, Cooker *et al.* (2002) reported that species that belong to the *Campylobacteraceae* are growing in their resistance against erythromycin, especially in developing countries. Findings from this study were higher than that reported for *Campylobacteraceae* isolates from chickens from South Africa (Simango, 2013), organic turkeys from Germany (Ahmed *et al.,* 2016) and cattle and sheep from Iran (Shirzad-Aski *et al.,* 2016). However, similar resistance levels to erythromycin have been seen in *Campylobacteraceae* species from chickens, cattle and sheep as resistance levels ranging from 63.6-100% have been reported (Karikari *et al.*, 2017; Yesilmen *et al.*, 2017). Furthermore, these

findings could be a potential indication that erythromycin might not be an effective line of defence in human infections induced by species that belong to the *Campylobacteraceae* family.

Tetracycline is a broad-spectrum antibiotic that is recommended for the treatment of campylobacteriosis. For both *Campylobacter* spp. (47.62%) and *Arcobacter* spp. (45.24%) it was found that tetracycline had the fourth highest resistance levels (Table 6.3). Previous research has indicated that species that belong to the *Campylobacteraceae* family obtained from food-producing animals can exhibit a high resistance against this antibiotic. For instance, Yesilmen *et al.* (2017) determined a 46-92% resistance for *Arcobacter* spp. and Karikari *et al.* (2017) determined a 60% resistance for *Campylobacter* spp. Research has attributed the high tetracycline resistance to the frequent use of this antibiotic at primary production (Simango, 2013; Raufu *et al.*, 2014). In fact, in the South African ostrich industry, ingoxytetracycline is used to treat clostridial enterotoxaemia in ostriches (Olivier, 2014), in which the active ingredient is tetracycline. Additionally, it is considered normal practice to treat ostriches exhibiting enteritis with tetracycline, as previously mentioned in Chapter 3. Therefore, misuse/overuse/prolonged use of medicine with tetracycline or tetracycline itself could have potentially contributed to the high resistance found in this study.

Overall, this study showed a high susceptibility (and in turn low resistance levels) towards quinolones (ciprofloxacin and nalidixic acid) as susceptibility levels ranging from 57.15 to 88.10% were determined for *Campylobacter* spp. and *Arcobacter* spp. isolates (Table 6.3). Lower resistance levels of 8.9-52.4% have also been reported for broiler chikens from South Africa (Bester & Essack, 2008) and livestock such as sheep and cattle from Iran (Shirzad Aski *et al.*, 2016) but, contradictory to El-Adawy *et al.* (2015) who found high levels of resistance to quinolones in organically reared turkeys from Germany. Research has attributed high resistance towards quinolones to excessive use in veterinary medicine (Rahimi & Ameri, 2011) and as a feed additive (Savaşan *et al.*, 2004). In fact, in South Africa, quinolones are used to treat bloody enteritis in ostriches (Olivier, 2014); the high susceptibility is an indication of the correct use/governance of these antibiotics. Even though the majority of the isolates were susceptible to ciprofloxacin, it is worrisome that some isolates showed resistance, as ciprofloxacin is seen as the first drug of choice for treating infections caused by species belonging to the *Campylobacteraceae* family (Shirzad-Aski *et al.*, 2016; Yoon *et al.*, 2017).

Campylobacter spp. and *Arcobacter* spp. isolates could be further classified as resistant to three or more antibiotic classes (multi-drug resistant; MDR). Multi-drug resistance was seen for most of the *Campylobacter* spp. (92.86%) and *Arcobacter* spp. (83.33%) isolates from ostriches (Fig. 6.2). Additionally, most of the *Campylobacter* spp. isolates were resistant to four antibiotic classes whilst most *Arcobacter* spp. isolates were resistant to three classes of antibiotics (Table 6.4). As reviewed by van den Honert *et al.* (2018), some microorganisms can become MDR due to the misuse of antibiotics at primary production. Microorganisms that are MDR harbour an array of genetic strategies that can effectively resist antibiotics. High rates of MDR in *Arcobacter* spp. and *Campylobacter* spp. isolates from food-producing animals has been reported ranging from 50 to 94% (Son *et al.*, 2007; Ibrahim *et al.*, 2013; Zhang *et al.*, 2018). The high rates of MDR can be considered an area of concern and warrants more research, as multi-

drug resistance can ultimately affect the choice of antibiotic therapy chosen to mitigate infections in humans (Goni *et al.*, 2018; Amador *et al.*, 2019).

6.6 Conclusion

The present study provides evidence of antibiotic resistance in *Campylobacter* spp. and *Arcobacter* spp. isolated from ostriches from South Africa and ostrich carcasses (and by extension, the susceptibility patterns are also presented). To the author's knowledge, this is the first study describing antibiotic resistance in *Campylobacter* and *Arcobacter* species from ostrich carcasses and ostriches from South Africa. *Campylobacter* spp. and *Arcobacter* spp. isolates were generally resistant to antibiotics in the following order cephalothin, vancomycin and erythromycin and just less than 50% of the *Campylobacter* spp. and *Arcobacter* spp. isolates were resistant to tetracycline. Additionally, the majority of the *Campylobacter* spp. and *Arcobacter* spp. isolates exhibited multi-drug resistance. This study provides evidence that species belonging to the *Campylobacteraceae* family sourced from ostriches, can pose a potential risk to consumer health and by extension this study illuminates the need to continually asses the efficacy of antibiotics typically administered to treat *Campylobacter* spp./*Arcobacter* spp. related infections in humans. Furthermore, this study highlights the need to investigate the use of essential antibiotics at primary production. If there is excessive use of antibiotics during the rearing process of ostriches, then results from the study could potentially help motivate the reduction of antibiotic use. However, it should be noted that a reduction or even a complete halt of the usage of antibiotics cannot completely eradicate the problem, as antibiotic resistance genes will not rapidly disappear from the gene pool, nonetheless it could aid in the decrease of the number of prevalent resistant bacteria.

6.7 References

Acheson, D. & Allos, B.M. (2002). *Campylobacter jejuni* Infections: Update on Emerging Issues and Trends. *Clinical Infectious Diseases*, **32**, 1201–1206.

Agyare, C., Boamah, V.E., Zumbi, C.N. & Osei F.B. (2018). Antibiotic use in poultry production and its effects on bacterial resistance. [Internet document]. URL https://www.intechopen.com/books/antimicrobial-resistance-a-global-threat/antibiotic-use-in-poultry-production-and-its-effects-on-bacterial-resistance 13/09/2019.

Ahmed, M.F.E.M., El-Adawy, H., Hotzel, H., Tomaso, H., Neubauer, H., Kemper, N., Hartung, J. & Hafez, H.M. (2016). Prevalence, genotyping and risk factors of thermophilic *Campylobacter* spreading in organic Turkey farms in Germany. *Gut Pathogens*, **8**, 1–9.

Altekruse, S.F. & Tollefson, L.K. (2003). Human campylobacteriosis: a challenge for the veterinary profession. *Journal of the American Veterinary Medical Association*, **223**, 445–452.

Amador, P., Fernandes, R., Prudêncio, C. & Duarte, I. (2019). Prevalence of antibiotic resistance genes in multidrug-resistant *Enterobacteriaceae* on Portuguese livestock manure. *Antibiotics*, **8**, 1-18.

Arguello, E., Otto, C.C., Mead, P. & Babady, N.E. (2015). Bacteremia caused by *Arcobacter butzleri* in an immunocompromised host. *Journal of Clinical Microbiology*, **53**, 1448–1451.

Baynes, R.E., Dedonder, K., Kissell, L., Mzyk, D., Marmulak, T., Smith, G., Tell, L., Gehring, R., Davis, J. & Riviere, J.E. (2016). Health concerns and management of select veterinary drug residues. *Food and Chemical Toxicology*, **88**, 112–122.

Brand, T. (2006). Volstruisvoedingsriglyne. In: *Volstruishandleiding* (edited by C. Muller). Pp. 42. Western Cape: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Brand, T. (2014). Ostrich nutrition guidelines. In: Ostrich manual (edited by P. Jorgensen). Pp. 13-23. Western Cape, Oudtshoorn: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Braykov, N.P., Eisenberg, J.N.S., Grossman, M., Zhang, L., Vasco, K., Cevallos, W., Muñoz, D., Acevedo, A., Moser, K.A., Marrs, C.F., Foxman, B., Trostle, J., Trueba, G. & Levy, K. (2016). Antibiotic Resistance in Animal and Environmental Samples Associated with Small-Scale Poultry Farming in Northwestern Ecuador. *mSphere*, **1**, 1–15.

Clinical and Laboratory Standards Institute. (2016a). Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria, 3rd ed. Method M45. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute.

Clinical and Laboratory Standards Institute. (2016b). Performance standards for antimicrobial susceptibility testing, 26th ed. Method M100S. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute.

Cogliani, C., Goossens, H. & Greko, C. (2011). Restricting antimicrobial use in food animals: Lessons from Europe. *Microbe*, **6**, 274–279.

Coker, A.O., Isokpehi, R.D., Thomas, B.N., Amisu, K.O. & Obi, C.L. (2002). Human campylobacteriosis in developing countries. *Emerging Infectious Diseases*, **8**, 237–243.

Collado, L. & Figueras, M.J. (2011). Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clinical Microbiology Reviews*, **24**, 174–192.

El-Adawy, H., Ahmed, M.F.E., Hotzel, H., Tomaso, H., Tenhagen, B.A., Hartung, J., Neubauer, H. & Hafez, H.M. (2015). Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* recovered from organic Turkey farms in Germany. *Poultry Science*, **94**, 2831–2837.

Fanelli, F., Pinto, A. Di, Mottola, A., Mule, G., Chieffi, D., Baruzzi, F., Tantillo, G. & Fusco, V. (2019). Genomic characterization of *Arcobacter butzleri* isolated from shellfish: Novel insight into antibiotic resistance and virulence determinants. *Frontiers in Microbiology*, **10**, 1–17.

Giacomelli, M., Salata, C., Martini, M., Montesissa, C. & Piccirillo, A. (2014). Antimicrobial Resistance of *Campylobacter jejuni* and *Campylobacter coli* from Poultry in Italy. *Microbial Drug Resistance*, **20**, 181–188.

Goni, M.D., Osman, A.Y., Aziz, S.A., Zunita, Z., Dhaliwal, G.K., Jalo, M.I., Bitrus, A.A., Jajere, S.M. & Abbas, M.A. (2018). Antimicrobial Resistance of *Campylobacter* spp. and *Arcobacter butzleri* from Pets in Malaysia. *American Journal of Animal and Veterinary Sciences*, **13**, 152–161.

Gouws, P. & Brozel, V. (2000). Antimicrobial resistance of *Salmonella* isolates associated with retail chicken and a poultry abattoir. *South African Journal of Sciences*, **96**, 254-256.

Ibrahim, M.J., Abdul-Aziz S., Bitrus A.A., Mohammed, D.G., Abu J., Bejo, S. K, Mohamed A.M., Mohamed, M.Y.I. (2013). Occurrence of multidrug resistant (MDR) *Campylobacter* species isolated from retail chicken meats in Selangor, Malaysia and their associated risk factors. *Malaysian Journal of Microbiology*, **9**, 111–115.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, **28**, 687–720.

Karikari, A.B., Obiri-Danso, K., Frimpong, E.H. & Krogfelt, K.A. (2017). Antibiotic Resistance of *Campylobacter* Recovered from Faeces and carcasses of healthy livestock. *BioMed Research International*, **2017**, 1–9.

King, T.L.B. & Schmidt, S. (2017). Assessment of three indigenous South African herbivores as potential reservoirs and vectors of antibiotic-resistant *Escherichia coli*. *European Journal of Wildlife Research*, **63**, 1-8.

Lastovica, A.J. (2006). Emerging *Campylobacter* spp.: The tip of the iceberg. *Clinical Microbiology Newsletter*, **28**, 49–56.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber J.T. & Monnet, D.L. (2011). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infectious Diseases*, **18**, 268-281.

Markets and Economic Research Centre (MERC) (2010). The South African Ostrich sub-sector. [Internet document]. URL http://www.namc.co.za/upload/all%20reports/Ostrich%20Value%20Chain%20Report.pdf02/10/2017.

Moore, J.E., Barton, M.D., Blair, I.S., Corcoran, D., Kempf, I., Lastovica, A.J., Dooley, J.S.G., Lowery, C.J., Matsuda, M., Mcdowell, D.A., Mcmahon, A., Millar, B.C., Rao, J.R., Rooney, P.J., Seal, B.S., Snelling, W.J. & Tolba, O. (2006). The epidemiology of antibiotic resistance in *Campylobacter*. *Mocrobes and Infection*, **8**, 1955-1966.

Olivier, A.J. (2014). Health management. In: Ostrich manual (edited by P. Jorgensen). Pp 86-107. Western Cape: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Rahimi, E. & Ameri, M. (2011). Antimicrobial resistance patterns of *Campylobacter* spp. isolated from raw chicken, turkey, quail, partridge, and ostrich meat in Iran. *Food Control*, **22**, 1165–1170.

Rahimi, E. (2014). Prevalence and antimicrobial resistance of *Arcobacter* species isolated from poultry meat in Iran. *British Poultry Science*, **55**, 174–180.

Raufu, I.A., Fashae, K., Ameh, J.A., Ambali, A.G., Ogunsola, F.T., Coker, A.O. & Hendriksen, R.S. (2014). Persistence of fluoroquinolone-resistant Salmonella enterica serovar Kentucky from poultry and poultry sources in Nigeria. *Journal of Infection in Developing Countries*, **8**, 384–388.

Saad, M.E., & Ahmed, M.B. (2016). Necessary usage of antibiotics in animals. [Internet document]. URL https://www.intechopen.com/books/antimicrobial-resistance-a-global-threat 09/13/2019.

Samie, A., Obi, C.L., Barrett, L.J., Powell, S.M. & Guerrant, R.L. (2007). Prevalence of *Campylobacter* species, Helicobacter pylori and *Arcobacter* species in stool samples from the Venda region, Limpopo, South Africa: Studies using molecular diagnostic methods. *Journal of Infection*, **54**, 558–566.

Savaşan, S., Çiftçi, A. & Diker, K.S. (2004). Emergence of quinolone resistance among chicken isolates of *Campylobacter* in Turkey. *Turkish Journal of Veterinary and Animal Sciences*, **28**, 391–397.

Sellu, D.P. (1986). *Campylobacter* enterocolitis: General and surgical aspects. *Postgraduate Medical Journal*, **62**, 719–726.

Shange, N., Gouws, P. & Hoffman, L.C. (2019). *Campylobacter* and *Arcobacter* species in food - producing animals : prevalence at primary production and during slaughter. *World Journal of Microbiology and Biotechnology*, **35**, 146.

Shirzad Aski, H., Tabatabaei, M., Khoshbakht, R. & Raeisi, M. (2016). Occurrence and antimicrobial resistance of emergent *Arcobacter* spp. isolated from cattle and sheep in Iran. *Comparative Immunology, Microbiology and Infectious Diseases*, **44**, 37–40.

Shobo, C.O., Bester, L.A., Baijnath, S., Somboro, A.M., Peer, A.K.C. & Essack, S.Y. (2016). Antibiotic resistance profiles of *Campylobacter* species in the South Africa private health care sector. *Journal of Infection in Developing Countries*, **10**, 1214–1221.

Simango, C. (2013). Antimicrobial susceptibility of *Campylobacter* species. *South Afr J Epidemiol Infect J Epidemiol Infect*, **28**, 139–142.

Soma, M.S., Tumati, S.R., Chinnam, B.K., Kothapalli, V.S. & Sharif, N.M. (2017). Virulence gene profiles of *Arcobacter* species isolated from animals, foods of animal origin, and humans in Andhra Pradesh, India. *Veterinary World*, **10**, 716–720.

Son, I., Englen, M.D., Berrang, M.E., Fedorka-Cray, P.J. & Harrison, M.A. (2007). Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *International Journal of Food Microbiology*, **113**, 16–22.

van den Honert, M.S., Gouws, P.A. & Hoffman, L.C. (2018). Importance and implications of antibiotic resistance development in livestock and wildlife farming in South Africa: A Review. *South African Journal of Animal Sciences*, **48**, 401–412.

Vandenberg, O., Dediste, A., Houf, K., Ibekwem, S., Souayah, H., Cadranel, S., Douat, N., Zissis, G. & Butzler, J. (2004). *Arcobacter* species in humans. *Emerging Infectious Diseases*, **10**, 1863-1867.

Vasoo, S., Schwab, J.J., Cunningham, S.A., Robinson, T.J., Cass, J.R., Berbari, E.F., Walker, R.C., Osmon, D.R. & Patel, R. (2014). *Campylobacter* prosthetic joint infection. *Journal of Clinical Microbiology*, **52**, 1771–1774.

Williams-Nguyen, J., Brett Sallach, J., Bartelt-Hunt, S., Boxall, A.B., Durso, L.M., McLain, J.E., Singer, R.S., Snow, D.D. & Zilles, J.L. (2016). Antibiotics and antibiotic resistance in agroecosystems: State of the science. *Journal of Environmental Quality*, **45**, 394–406.

Witte, W. (1998). Medical Consequences of Antibiotic Use in Agriculture. *I SCIENCE ' S COM PASS*, **279,** 996-997.

Yamana, T. & Tsuji, A. (1976). Comparative Stability of Cephalosporins in Aqueous soulution: Kinetics and mechanisms of degradation. *Pharmaceutical Sciences*, **65**, 1563-1574.

Yesilmen, S., Vural, A., Erkan, M.E. & Yildirim, I.H. (2017). Isolation and determination of antimicrobial resistance

of *Arcobacter* species isolated from animal faeces in the Diyarbakir region of Turkey using the 16S rDNA-RFLP method. *Veterinarni Medicina*, **62**, 301–307.

Yoon, J.G., Lee, S.N., Hyun, H.J., Choi, M.J., Jeon, J.H., Jung, E., Kang, S., Kim, J., Noh, J.Y., Choi, W.S., Song, J.Y., Cheong, H.J. & Kim, W.J. (2017). *Campylobacter jejuni* bacteremia in a liver cirrhosis patient and review of literature: A case study. *Infection and Chemotherapy*, **49**, 230–235.

Zhang, X., Tang, M., Zhou, Q., Zhang, J., Yang, X. & Gao, Y. (2018). Prevalence and characteristics of *Campylobacter* throughout the slaughter process of different broiler batches. *Frontiers in Microbiology*, **9**, 1–9.

Zhang, Y., Marrs, C.F., Simon, C. & Xi, C. (2009). Wastewater treatment contributes to selective increase of antibiotic resistance among *Acinetobacter* spp. *Science of the Total Environment*, **407**, 3702–3706.

CHAPTER 7

General discussion, conclusions and recommendations

Ostrich meat is an important commodity to the South African ostrich industry. Ostrich meat is sold to the local market and also exported to various countries in the world (MERC, 2010). It is therefore imperative to provide international and local consumers with ostrich meat that is safe for human consumption. The safety of ostrich meat can be compromised by emerging pathogens such as *Campylobacter* spp. and *Arcobacter* spp.; collectively belonging to the *Campylobacteraceae* family. Species belonging to the *Campylobacteraceae* family, have been implicated in the cause of the gastrointestinal infections in humans and more severe illnesses such as Guillian Barré Syndrome, bacteraemia and septicaemia (Collado & Figueras, 2011; Kaakoush *et al.*, 2015). Species belonging to the *Campylobacteraceae* family are harboured in the gastrointestinal tract of food-producing animals, where poultry species are seen as the primary reservoir and the consumption of poultry meat and meat products is recognised as the most likely route of infection for humans (Shange *et al.*, 2019). Currently, there is a lack of information that highlights whether ostriches from South Africa harbour *Campylobacter* and *Arcobacter* species. Therefore, the overall aim of this dissertation was to determine the prevalence of *Campylobacter* and *Arcobacter* species in ostriches from South Africa.

The first aim of this study was to investigate the onset of *Campylobacter* and *Arcobacter* spp. colonisation in artificially and naturally reared ostrich chicks and subsequently evaluate the prevalence of *Campylobacter* spp. and *Arcobacter* spp. in slaughter-aged ostriches. Previous research has indicated that *Campylobacter* spp. infection in poultry chicks can be stunted due to the presence of maternal antibodies and the intestinal flora during the early stages of life, therefore colonisation is unlikely to occur before 2-3 weeks of age (Van der Wielen *et al.*, 2000; Allen *et al.*, 2011). However, after the first 2-3 weeks of life colonisation was expected, as seen for broiler chickens and turkeys (Berndtson *et al.*, 1996; Saleha, 2004; El-Adawy *et al.*, 2012). A delayed onset was witnessed for *Campylobacter* spp., as *Campylobacter* spp. (*C. jejuni*) was only detected on the 12th week for intensively reared ostrich chicks. The delayed onset was partly attributed to farm practices. Simply put, it could be postulated that when ostrich chicks were moved from housing facilities to grazing camps at the age of 12 weeks, this act created an opportunity for colonisation. Furthermore, tetracycline was administered to the ostrich chicks used for this study. Previous research has shown that some *Campylobacter* spp. strains from South Africa could be susceptible to tetracycline (Simango, 2013). A persistent presence of *Arcobacter* spp. was determined for *Arcobacter* spp. from the beginning of sampling until the end (from the age of 2 to 12 weeks). The consistent detection of *Arcobacter* spp. could be an indication of a consistent source of *Arcobacter* spp. at a typical ostrich chick farm. Furthermore, in Chapter 3, cohorts of sacrificed ostrich chicks were moved to another farm and were sampled at the slaughter age of 10 months and 12 months. This part of the study showed that once an infection has entered a flock, it can possibly prevail until the age of slaughter at high prevalence levels of 56 to 70% for species belonging to the *Campylobacteraceae* family. Before this study it was not evident,

whether ostriches from South Africa were reservoirs of *Arcobacter* spp. Therefore, the successful detection shows that ostriches can be reservoirs of *Campylobacter* and *Arcobacter* species.

The aim of the second study was to determine the prevalence levels of both *Campylobacte*r and *Arcobacter* species in live ostriches reared on South African farms. Prevalence of *Campylobacter* spp. and *Arcobacter* spp. was determined by sourcing cloacal swabs (n = 836) from ostriches reared in 30 different farms situated in the Oudtshoorn region. PCR determined an average prevalence of 24.63% for species belonging to the *Campylobacteraceae* family. The ISO 10272-1:2006 method determined a *Campylobacter* spp. prevalence level of 16.83%, whilst the Cape Town protocol could not detect *Campylobacter* spp. For *Arcobacter* spp., a prevalence of 18.80% and 39.14% was determined with the Cape Town protocol and the selective *Arcobacter* spp. method, respectively. This study showed that higher prevalence levels could be detected with the use of longer enrichment periods, thus selective methods which made use of longer enrichment periods allowed for more effective resuscitation of injured and stressed *Campylobacter* spp. and *Arcobacter* spp. cells (Hayashi *et al.*, 2013). With this study, it was also seen that prevalence levels could be influenced by season, water sources and the presence of wild water birds on farm premises. A higher prevalence of *Campylobacter* spp. and *Arcobacter* spp. was seen during the warmer spring months and the cooler autumn months, respectively. Prevalence levels were higher when ostriches consumed borehole water. Furthermore, when farm premises were prone to the presence of wild water birds higher prevalence levels were seen, and this could be attributed to the fact that contaminated water sources and wild water birds can potentially aid in horizontal transmission (Olivier, 2014; Giacometti *et al.*, 2015) resulting in higher prevalence levels.

The aim of the third study was to determine possible points of contamination and occurrence of *Campylobacter* and *Arcobacter* species during slaughter. This objective was achieved by following the slaughter of ostriches infected with *Campylobacter* and *Arcobacter* species, as determined in Chapter 4. The study confirmed what has been expressed by other researchers, that *Campylobacter* spp. and *Arcobacter* spp. can be transferred to sterile carcasses during the skinning and evisceration process (Shange *et al.*, 2019). Furthermore, this study also corroborated a trend again seen in literature that the occurrence of *Campylobacter* spp. and *Arcobacter* spp. contamination can possibly be reduced by control measures such as the chilling (De smet *et al.*, 2010) of carcasses for 24 hours (0 - 4ºC). The second part of this study determined the presence of both *Campylobacter* spp. and *Arcobacter* spp. in weekly samples obtained from three abattoirs situated in the Western and Eastern Cape region, as well as determined faecal contamination during the slaughter of ostriches. Literature places special emphasis on faecal contamination of carcasses, as this is recognised as the mostly likely route in which *Campylobacter* spp. and *Arcobacter* spp. can be transferred to carcasses during slaughter (Shange *et al.*, 2019). Previous research has indicated that faecal indicator microorganisms can have a strong correlation with species belonging to the *Campylobacteraceae* family, such as increasing after evisceration and decreasing after chilling (Pacholewicz *et al.*, 2016). However, with the low occurrences of *Campylobacter* spp., *Arcobacter* spp., and faecal indicator microorganisms (*E. coli* and coliforms) found in this study, a correlation could not be proven. Overall, this was an attempt to determine the occurrence of *Campylobacter* spp. and *Arcobacter* spp. if abattoirs were

required to test samples on a regular basis. This study showed that under good hygienic practices, it is possible to obtain ostrich carcasses with high microbiological standards as low levels of *Campylobacter* spp., *Arcobacter* spp. *E. coli* and coliforms were determined. Specific regulations for the presence of *Campylobacter* spp. and *Arcobacter* spp. on ostrich meat and/or carcasses do not exist in South Africa. However, the isolation of *Campylobacter* spp. and *Arcobacter* spp. during routine testing (second study) potentially advocates for routine testing of species that belong to the *Campylobacteraceae* family. In saying, that more research is required to determine the frequency in which sampling should occur.

The aim of the fourth study was to determine antibiotic resistance of *Campylobacter* spp. and *Arcobacter* spp. isolates obtained from ostriches and ostrich meat. Resistance was determined in the following order: cephalothin and vancomycin, erythromycin, tetracycline, nalidixic acid and ciprofloxacin. The highest susceptibility for *Campylobacter* spp. and *Arcobacter* isolates was seen for nalidixic acid and ciprofloxacin. Furthermore, multi-drug resistance was determined for most of the *Campylobacter* spp. and *Arcobacter* spp. isolates. This study corroborated the notion that species belonging to the *Campylobacteraceae* family are growing in their resistance to essential antibiotics and multidrug resistance (MDR) (Son *et al.*, 2007; Simango, 2013; Giacomelli *et al.*, 2014). These results can potentially contribute to the notion that a more prudent use of essential antibiotics is needed, as resistance and multi-drug resistance might affect antibiotic therapy administrated to severe cases of gastrointestinal infections caused by *Campylobacter* spp. and *Arcobacter* spp.

Following the results obtained from this study and limitations experienced during the study some recommendations have been made for future studies. Findings from Chapter 3 and 4 highlight a need to include the sampling of environmental sources (such as water sources, soil and wild water birds) and possibly determine genotypic relatedness between isolates obtained from the environmental sources and ostriches. Furthermore, samples from ostrich chick farms, ostrich farms and ostrich abattoirs situated in other parts South Africa could contribute to findings, as in Chapter 3, 4 and 5 most of the samples were obtained from the Western Cape. Furthermore, as contamination can occur during the slaughter process (Chapter 5) it could potentially be worthwhile to determine the pathogenicity of *Campylobacter* spp. and *Arcobacter* species from ostriches, as well as the genes responsible for antibiotic resistance.

Additionally, even though this study detected the presence of *Campylobacter* and *Arcobacter* species, the load ($\log_{10}$ cfu/g) in which these organisms are carried by ostriches and the load ($\log_{10}$ cfu/g) in which contamination occurs could not be investigated. If in future studies, immediate testing can be executed (before reaching a viable but not culturable state (VBNC) occurs) *Campylobacter* spp. and *Arcobacter* spp. should be enumerated. With this approach flocks could be classed according to their microbial load and tested accordingly. In turn this data would help shape future microbial specifications (sampling frequency, upper and lower limits) for *Campylobacter* spp. and *Arcobacter* spp., which could be included in important documents such as VPN 52/2018.

7.1 References

Allen, V.M., Ridley, A.M., Harris, J.A., Newell, D.G. & Powell, L. (2011). Influence of production system on the rate of onset of *Campylobacter* colonization in chicken flocks reared extensively in the United Kingdom. *British Poultry Science*, **52**, 30–39.

Berndtson, E., Danielsson-Tham, M.L. & Engvall, A. (1996). *Campylobacter* incidence on a chicken farm and the spread of *Campylobacter* during the slaughter process. *International Journal of Food Microbiology*, **32**, 35–47.

Collado, L. & Figueras, M.J. (2011). Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clinical Microbiology Reviews*, **24**, 174–192.

De Smet, S., Zutter, L. De, Hende, J. Van & Houf, K. (2010). *Arcobacter* contamination on pre- and post-chilled bovine carcasses and in minced beef at retail. *Journal of Applied Microbiology*, **108**, 299–305.

El-Adawy, H., Hotzel, H., Tomaso, H., Neubauer, H. & Hafez, H.M. (2012). Elucidation of colonization time and prevalence of thermophilic Campylobacter species during turkey rearing using multiplex polymerase chain reaction. *Poultry Science*, **91**, 454–459.

Giacomelli, M., Salata, C., Martini, M., Montesissa, C. & Piccirillo, A. (2014). Antimicrobial Resistance of *Campylobacter jejuni* and *Campylobacter coli* from Poultry in Italy. *Microbial Drug Resistance*, **20**, 181–188.

Giacometti, F., Lucchi, A., Francesco, A. Di, Delogu, M., Grilli, E., Guarniero, I., Stancampiano, L., Manfreda, G., Merialdi, G. & Serraino, A. (2015). Arcobacter butzleri, Arcobacter cryaerophilus, and Arcobacter skirrowii circulation in a dairy farm and sources of milk contamination. *Applied and Environmental Microbiology*, **81**, 5055–5063.

Hayashi, M., Kubota-Hayashi, S., Natori, T., Mizuno, T., Miyata, M., Yoshida, S., Zhang, J., Kawamoto, K., Ohkusu, K., Makino, S. & Ezaki, T. (2013). Use of blood-free enrichment broth in the development of a rapid protocol to detect *Campylobacter* in twenty-five grams of chicken meat. *International Journal of Food Microbiology*, **163**, 41–46.

ISO 10272:1 (2006). Microbiology of food and animal feeding stuffs horizontal method for detection and enumeration of *Campylobacter* spp. URL https://www.iso.org/standard/37091.html. 03/01/2017.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, **28**, 687–720.

Markets and Economic Research Centre (MERC), (2010). The South African Ostrich sub-sector. [Internet document]. URL http://www.namc.co.za/upload/all%20reports/Ostrich%20Value%20Chain%20Report.pdf 02/10/2016.

Olivier, A.J. (2014). Health management. In: Ostrich manual (edited by P. Jorgensen). Pp 86-107. Western Cape: Western Cape Department of Agriculture and South African Ostrich Business Chamber.

Pacholewicz, E., Swart, A. & Havelaar, A.H. (2016). Explanatory variables associated with *Campylobacter* and *Escherichia coli* concentrations on broiler chicken carcasses during processing in two slaughterhouses. *Journal of Food Protection*, **79**, 2038-2047.

Saleha, A.A. (2004). Epidemiological study on the colonization of chickens with *Campylobacter* in broiler farms in Malaysia: Possible risk and management factots. *International Journal of Poultry Science*, **3**, 129–134.

Shange, N., Gouws, P. & Hoffman, L.C. (2019). *Campylobacter* and *Arcobacter* species in food - producing animals: prevalence at primary production and during slaughter. *World Journal of Microbiology and Biotechnology*, **35**, 146.

Simango, C. (2013). Antimicrobial susceptibility of *Campylobacter* species. *South Afr J Epidemiol Infect J Epidemiol Infect*, **28**, 139–142.

Son, I., Englen, M.D., Berrang, M.E., Fedorka-Cray, P.J. & Harrison, M.A. (2007). Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *International Journal of Food Microbiology*, **113**, 16–22.

Van der Wielen, P., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B. & Knapen, F. (2000). Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Applied and Environmental Microbiology*, **66**, 2536–2540.

Veterinary Procedural Notices (VPN) 52 (2018). Guidelines on the verification of compliance with process hygiene control and microbiological food safety performance objectives under the Meat Safety Act. URL at http://www.rmaa.co.za/wp-content/uploads/2018/12/VPN-52-Microbiological-Reference-Criteria-for-Meat-19-October-2018-Draft.pdf. 5/08/2019.